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FOR

CHLOROBENZENE

FINAL DRAFT

June 1988

CRITERIA AND STANDARDS DIVISION

OFFICE OF DRINKING WATER

U.S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

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FOREWORD

Section 1412(b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is non-enforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity were evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document was comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature search in support of this document includes information published up to 1987; however, more recent information may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (One-day, Ten-day and Longer-term, approximately 10% of an individual's lifetime) are included in this document. These values are not used in establishing the MCLG but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

Michael B. Cook
Director
Office of Drinking Water

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The Office of Drinking Water (ODW), Environmental Protection Agency has prepared a Drinking Water Criteria Document on Monochlorobenzene. This Criteria Document is an extensive review of the following topics:

- Physical chemical properties of Monochlorobenzene
- Toxicokinetics and human exposure to Monochlorobenzene
- Health Effects of Monochlorobenzene in humans and animals
- Mechanisms of toxicological effect of Monochlorobenzene
- Quantification of toxicological effects of Monochlorobenzene

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TABLE OF CONTENTS

		Page
I.	SUMMARY	I-1
II.	GENERAL INFORMATION AND PROPERTIES	II-1
III.	TOXICOKINETICS	III-1
IV.	SOURCES OF HUMAN EXPOSURE*	IV-1
V.	HEALTH EFFECTS IN ANIMALS	V-1
VI.	HEALTH EFFECTS IN HUMANS	VI-1
VII.	MECHANISM(S) OF TOXICITY	VII-1
VIII.	QUANTIFICATION OF TOXICOLOGICAL EFFECTS	VIII-1
IX.	REFERENCES	IX-1

*Prepared by the Science and Technology Branch

I. SUMMARY

Chlorobenzene has been used industrially in great quantities. It has been detected in raw water sources, waste waters and even in finished drinking water supplies.

Chlorobenzene is poorly soluble in water but highly soluble in lipids. It is absorbed readily from the lungs, gastrointestinal tract and, to a lesser degree, through the skin. Chlorobenzene is metabolized *in vivo* principally to 4-chlorocatechol and 4-chlorophenol. The metabolites are eliminated from the body in bile, feces, urine and expired air. The phenolic metabolites are excreted mainly as conjugates of glucuronide and sulfate conjugates and as mercapturic acids.

Biodegradation as well as chemical and physical degradation of chlorobenzene in the environment tends to be slow.

Chlorobenzene has been shown to be toxic to various aquatic species, including fish and other aquatic organisms consumed by humans, microorganisms, insects, a variety of laboratory animals and humans. Acute, high dose effects of these compounds include sedation, anesthesia and death due to respiratory failure. Apparent central nervous system (CNS) stimulation may precede CNS depression. Chronic exposure to these substances may result in blood dyscrasias and lung, liver and kidney damage. Porphyria due to effects on porphyrin synthesis often accompanies the liver damage.

Reports of reproductive and/or teratogenicity studies have been found. Dow Chemical Company has completed teratology studies

of chlorobenzene in rats and rabbits. In the Phase I studies, there was no evidence of teratological changes in the rats, but there were some suggestive changes in the rabbits at the highest dose tested. The experiments with the rabbits were repeated in the Phase II study. No teratogenic effects were seen during this experiment. In a recent study (Nair et al., 1987), inhalation of CB by rats did not affect reproductive performance, but treatment-related pathology was evident with the two highest exposure levels.

Chlorobenzene has been tested in numerous mutagenicity assays with mixed results being obtained; the weight of evidence suggests chlorobenzene has low potential for mutagenic effects. A technical report on the carcinogenesis bioassay of chlorobenzene (gavage studies in F344 rats and B6C3F1 mice) states that, under the conditions of the bioassay, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in the high dose male rats, providing some but not clear evidence of carcinogenic activity in male rats (NTP, 1983). Following evaluation of these data, the EPA Carcinogen Assessment Group determined that it was inappropriate to derive a carcinogenic potency factor or estimated excess lifetime cancer risks. The EPA has classified chlorobenzene as Group D: Inadequate evidence. The EPA's Science Advisory Board has concluded that the carcinogenicity evidence for chlorobenzene leads to a classification in Group D, in which the evidence in animals and humans is inadequate for making conclusions on carcinogenicity.

Because there are inadequate data for derivation of One-day and Ten-day Health Advisories, the Longer-term Health Advisory for

the 10-kg child is recommended for use as conservative One-day and Ten-day Health Advisories. Longer-term Health Advisories of 1.9 mg/l for a 10-kg child and 6.8 mg/l for a 70-kg adult are derived. Using a reference dose (RfD) of 0.02 mg/kg/day, a drinking water equivalent level (DWEL), where 100% exposure to chlorobenzene from drinking water is assumed, of 0.7 mg/l is calculated. The DWEL may be further reduced to take into account relative source contribution.

II. GENERAL INFORMATION AND PROPERTIES

Chemical and Physical Properties

At 20°C, chlorobenzene (CB) is a colorless, neutral liquid of molecular weight 112.6, with a distinctive odor of almonds. It is relatively insoluble in water (490 mg/l), but miscible in all proportions with many organic liquids, such as alcohol, benzene, the chlorinated solvents, ethers and aliphatic and aromatic hydrocarbons (Irish, 1963). At 25°C, CB has a specific gravity of 1.106 and a vapor pressure of 11.8 mm Hg. It solidifies at -45°C and boils at 132°C. It forms an azeotrope with water which boils at 90°C (71.6% chlorobenzene, 28.4% water, by weight). Since it is slightly heavier than water, it may sink to the bottom of still bodies of water.

In air, 1 ppm CB = approximately 4.6 mg/m^3 and 1 mg/l = 217 ppm at 25°C, 760 mm Hg (Irish, 1963).

Sato and Nakajima (1979) determined partition coefficients for CB. While the water/air coefficient was low (4.1), the oil/air coefficient was quite high (3,763). The blood/air coefficient was 30.8. In addition, the oil/water coefficient was 918. Thus, chlorobenzene is quite lipophilic (log P = 2.96).

Additional evidence suggests a moderate potential for bioconcentration. Leo, et al. (1971) reported an octanol/water partition coefficient of about 700. Lu and Metcalf (1975) studied the environmental fate of and biodegradability of CB in a model ecosystem containing Daphnia, mosquito larvae, snails, fish and green algae. The data indicated that considerable ecological

magnification (EM) occurred. EM values ranged from 645 for fish to 4,200 for algae.

Human thresholds for detection of chlorobenzene have been reported. The odor and taste threshold for CB in water has been reported to range from 0.45-1.5 ug/l (Tarkhova, 1965) to 10-20 ug/l (Varshavskaya, 1968). A.D. Little (1968), in a report to the Manufacturing Chemists Association, listed the odor threshold as 0.21 mg/l (medium not designated). None of these citations described the experimental conditions/protocols used in the determination of these thresholds. Therefore, it is difficult to interpret these data, judge their validity or account for the disparity in the findings of the investigators.

Production and Use

Chlorobenzene is used in significant quantities in this country. U.S. production in 1977 was about 150,000 kkg (the most recent data available to the Office of Drinking Water) (U.S. ITC, 1978). Imports accounted for nearly 500 kkg. At that time, approximately 35% of the chlorobenzene was utilized in the production of chloronitrobenzenes, 10% in diphenyl oxide production, 10% for rubber intermediates and 7% for DDT. About 30% of the annual production volume was used as a solvent in adhesives, paints, waxes, and polishes and as an inert process solvent.

III. TOXICOKINETICS

Absorption/Retention

Although there apparently have been no studies of the systemic absorption of chlorobenzene (CB), it should be well absorbed since it is highly lipid soluble. Because of this property, it can readily traverse many tissue barriers *in vivo*, such as the placenta and blood:brain barrier. It is absorbed from the lungs, the gastrointestinal tract and through the skin.

No data are available which demonstrate the percentage of a dose absorbed following exposure to CB in any medium. Based upon what is known about the absorption characteristics of benzene and the smaller chlorinated aliphatics (ethanes and ethylenes), it will be assumed that 100% of any oral dose is absorbed, while 50% of a dose inhaled over a period of one to several hours is absorbed and retained (Astrand, 1975; Dallas, *et al.*, 1983).

Distribution

A distribution study has been done in male Sprague-Dawley rats following inhalation exposure (Sullivan, *et al.*, 1983). Groups of 15 animals each were exposed at 100, 400 or 700 ppm ^{14}C-chlorobenzene (460, 1,840 or 3,220 mg/m^3, respectively) for 8 hours. Six of each group were exposed for 5 consecutive days. Another 3 were exposed for the first four days of the multiple exposure regimen, but then killed on the morning of the fifth day, 16 hours after their last exposure. The remaining 6 rats were exposed only on the fifth day for 8 hours. Liver, lung, kidney, blood, epididymal and perirenal fat were analyzed for

^{14}C content. As might be expected, the highest tissue burdens were found in the fat (epididymal and perirenal). Kidneys and liver also showed significant amounts. The amounts found in these tissues were proportional to dose except for the adipose tissue which showed greatly exaggerated accumulation with dose when compared to the other tissues. The ^{14}C burden of adipose tissue increased 8- to 10-fold when the exposure concentration increased from 100 to 400 ppm and 3- to 5-fold when the exposure increased from 400 to 700 ppm. Also, there was a tendency for rats with multiple exposures to exhibit higher tissue burdens than rats exposed only once.

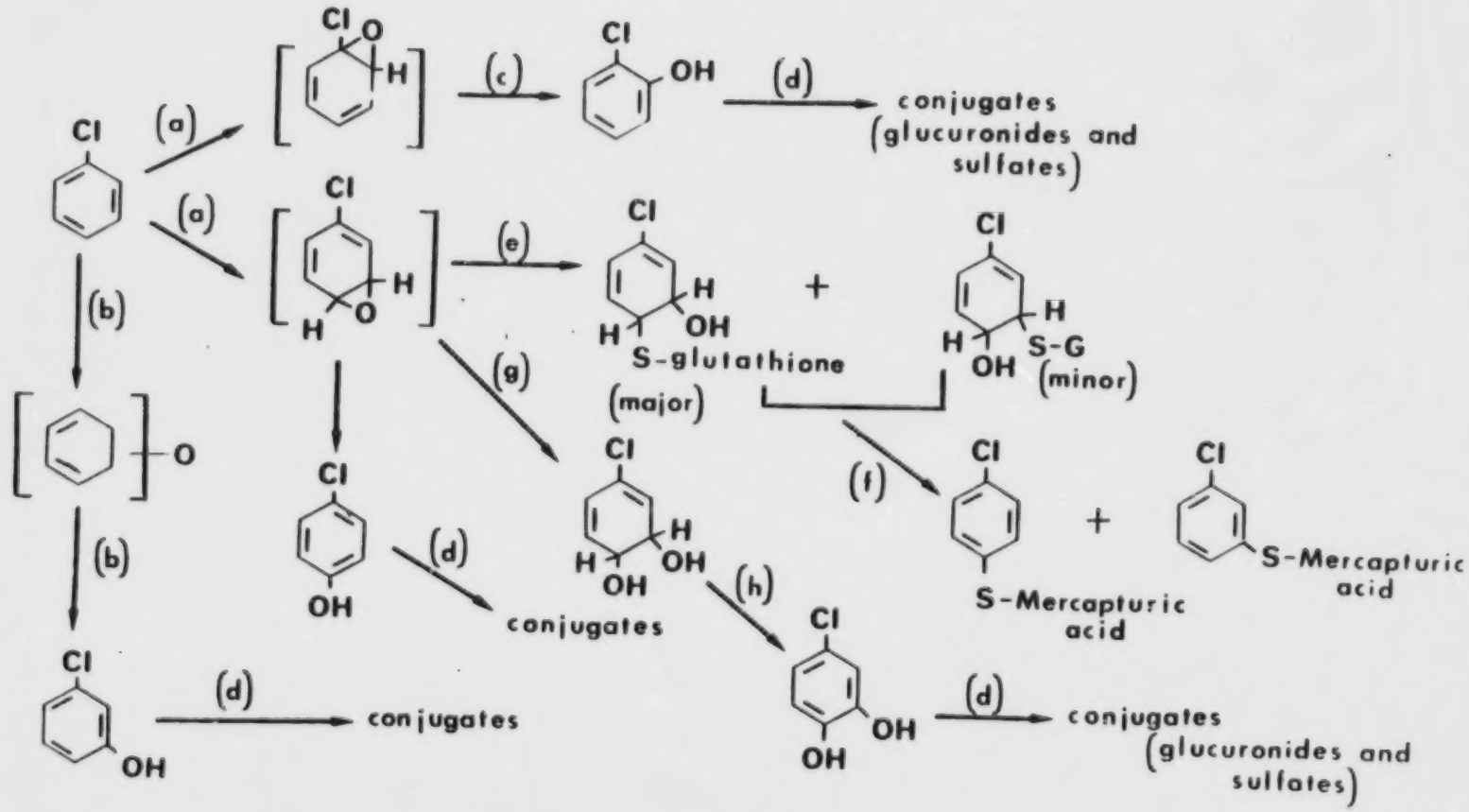
Metabolism

Figure III-1, kindly provided by the Chlorinated Solvents Subcommittee of the Environmental Health Committee of the U.S. EPA Science Advisory Board which reviewed this document, depicts the metabolites of CB identified and the metabolic pathways proposed. The several reported studies providing assessments of metabolism include Williams (1959); Lindsay-Smith, et al. (1972); Selander, et al. (1975); Shimada (1981); and Sullivan (1981).

Auret, et al. (1984) noted that metabolism of chlorobenzene in liver systems has been widely investigated. They indicated that the intermediacy of dihydroarene 3,4-oxides of chlorobenzene has been determined with ^{14}C-chlorobenzene and chemically synthesized 1-chloro-3,4-dihydrobenzene 3,4-oxide. They stated that it has not proved possible to distinguish between involvement of either dihydroarene 1,2-oxide or 2,3-oxide metabolites during

FIGURE III-1. Metabolism of Monochlorobenzene

Provided by the Chlorinated Solvents Subcommittee of the Environmental Health Committee of the U.S. EPA Science Advisory Board.



(a) mixed function oxidases (MFO), NADPH, O_2; (b) unknown mechanism; (c) spontaneous rearrangement; (d) glucuronosyl transferases/UDPGA and sulfotransferases/PAPS; (e) glutathione transferases/GSH; β dehydration, peptidase, acetyltransferase yields mercapturic acid; (g) exporide hydrolase/H_2O; (h) dehydrogenase ($-H_2$)

NOTE: Additional oxidation of phenols and catechols and dioxygenase ring cleavage can also occur.

mammalian metabolism of chlorobenzene to form ortho-halogenphenols. In their studies with fungi, Auret, et al. (1984) found evidence for initial formation of dihydroarene oxides during ortho-hydroxylation of chlorobenzene. Although they found evidence that enzyme-catalyzed epoxidation occurs preferentially at the 2,3-bond of chlorobenzene in fungi, they also felt that there is presently no justification for extending this conclusion to metabolism of chlorobenzene in mammalian liver.

After a single 0.5 mg/kg oral dose of CB administered to rabbits, 27% was excreted unchanged in expired air within 1 to 2 days (Spencer and Williams, 1950; Azouz, et al., 1953). Some was excreted unchanged in the urine. The remaining portion was metabolized in vivo. Following conversion to a 3,4-arene oxide (A), CB was further metabolized via glutathione conjugation to chlorophenyl-mercapturic acid (B), via hydroxylation to o- and/or p-chlorophenol (C), or to p-chlorocatechol (E) from 3,4-dihydro-3,4-dihydroxychlorobenzene (D). Small amounts of phenol (F), hydroquinone (G) and some diphenols have been detected in the urine of some species.

Shimada (1981) reported the occurrence of p-chlorophenyl mercapturic acid as well as monoglucuronide and ethereal sulfate conjugates of 4-chlorocatechol in the urine of rats given chlorobenzene (dose not stated) by subcutaneous injection. He proposed a metabolic pathway in which monochlorobenzene is initially oxidized to 4-chlorobenzene-1,2-epoxide which, in turn, may: (1) form a glutathione conjugate, resulting in excretion of p-chlorophenyl mercapturic acid; (2) be converted to 4-chlorophenol,

conjugated and excreted; or (3) be converted to 4-chlorocatechol, conjugated and excreted.

Using perfused rat livers and cell-free liver preparations, Selander, et al. (1975) found that chlorobenzene is converted to chlorophenols by three different enzymes, two of which formed arene oxide intermediates (3- and 4-chlorobenzene oxides) resulting in the formation of o- and p-chlorophenol. m-Chlorophenol appeared to be formed by direct oxidation.

After gavage administration of 14C-chlorobenzene emulsified in Cremophor E.L. and physiological saline to two adult female Dutch rabbits twice daily for four days, Smith, et al. (1972) identified the following metabolites as over 98% of urinary activity: 3,4-dihydro-3,4-dihydroxychlorobenzene (0.57%); monophenols (2,84%); diphenols (4.17%); mercapturic acids (23.8%); ethereal sulfates (33.88%); and glucuronides (33.57%).

Ogata and Shimada (1982) compared chlorobenzene metabolism in rats, dosed orally or intraperitoneally, and humans dosed orally. Polyethylene glycol was the dosing vehicle. Urine was also obtained from workers occupationally exposed to chlorobenzene. The main urinary metabolite in rats was p-chlorophenyl mercapturic acid. In humans, conjugates of 4-chlorocatechol were the primary urinary metabolites.

Dalich and Larson (1985) reported depression of cytochrome P-450 levels to 50-80% of control levels, with no clear dose-response, after treatment of male Sprague-Dawley rats (75-125 g) with chlorobenzene at single intraperitoneal doses of 2.0-14.7 mmol/kg (225.2-1,655.2 mg/kg).

Lindsay-Smith, et al. (1972) found that the conjugated metabolites were both mono- and diphenolic in the rabbit, but the monophenolics were predominant. p-Monochlorophenol was the predominant isomer in the urine. The distribution of isomers for free and conjugated monochlorophenol combined was: ortho-, 4.9%; meta-, 22.9%; and para-, 72.2%. For free monochlorophenols, the distribution of isomers was 5.9, 33.6 and 60.4% for ortho-, meta- and para- isomers, respectively. The major diphenolic metabolite was reported to be 4-chlorocatechol; small amounts of chloroquinol, 3-chlorocatechol and quinol also were found. Although there was not adequate proof in these studies, it was proposed that metabolism proceed through the formation of an arene oxide (3,4-chlorobenzene oxide). Conjugation of this arene oxide with glutathione followed by further metabolic reactions would account for the meta- and para-chlorophenyl mercapturic acids but not the ortho- isomer. Hydration of the arene oxide, followed by dehydrogenation, would lead to chlorocatechol.

The metabolism of ^{14}C-chlorobenzene in thirteen mammalian species (as described by Williams, et al., 1975) is shown in Table III-1. The basic pattern of metabolites appears to be similar, although quantitatively different, from one species to another. For example, p-chlorophenyl mercapturic acid formation is high in the hedgehog, but low in the guinea pig. Man appears to be similar to the guinea pig in this respect.

It also might be noted that the fate of ^{14}C-chlorobenzene in the rat is dose dependent (Sullivan, et al., 1983). As the dose is increased, the percentage expired in the air increases

Table III-1. Metabolism of ^{14}C-Chlorobenzene in Different Species (Williams, et al., 1975)

Species	% of 24-hour excretion of ^{14}C as: p-Chlorophenol	p-Chlorocatechol	p-Chlorophenyl-mercapturic acid
Man	33	31	19
Rhesus monkey	19	37	40
Squirrel monkey	14	37	50
Capuchin monkey	19	36	41
Dog	14	45	42
Ferret	33	31	24
Hedgehog	20	12	65
Rabbit	29	38	26
Rat	23	22	49
Mouse	20	31	42
Gerbil	13	26	51
Hamster	15	23	43
Guinea pig	27	35	21

and that in the urine decreases (Table III-2). There also is a shift in the urinary metabolites in the rat with a decrease in the mercapturic acids and an increase in the sulfates and glucoronides (Table III-3).

Excretion

The principal routes of excretion for chlorobenzene are exhalation and urine.

The ratio of unchanged to metabolized compound increases with increasing dose. The rate of respiratory excretion was evaluated by expressing the data for each animal as the percentage of total amount available for respiratory elimination which remained to be excreted at any time (t) post-exposure (Sullivan, et al., 1983). It was assumed that 100% remained 0 hours post-

Table III-2. Urinary and Respiratory Excretion of ^{14}C in Rats Exposed to Chlorobenzene by Inhalation[a] (Sullivan, et al., 1983)

Exposure concentration (ppm)	Exposure regimen[b]	Percentage excreted in urine	Percentage excreted in respiration	Total micromoles excreted ± SE
100	S	94.9 ± 0.4	5.1 ± 0.4	62.1 ± 6.7
100	M	97.1 ± 0.3[c]	2.9 ± 0.3[c]	76.6 ± 13.1
400	S	78.7 ± 2.3[d]	21.3 ± 2.3[d]	224 ± 4[d]
400[e]	S	74.5 ± 2.8[d]	25.5 ± 2.8[d]	281 ± 15[d]
400	M	86.9 ± 0.4[c,d]	13.1 ± 0.4[c,d]	212 ± 23[d]
700	S	55.7 ± 4.1[f]	44.3 ± 4.1[f]	774 ± 48[f]
700	M	68.3 ± 0.5[c,f]	31.7 ± 0.5[c,f]	639 ± 22[c,f]

a $\bar{x}$ ± SE for n = 3 rats, except in the repeat 400-ppm study, n = 6. Percentages are based on the amount excreted within 48 hr after exposure, not on total dose.

b S = single exposure, M = multiple exposure.

c Significantly different from single exposure ($p < 0.05$).

d Significantly different from 100- and 700-ppm groups ($p < 0.05$).

e Data from the repeat 400-ppm exposure block.

f Significantly different from 100- and 400-ppm groups ($p < 0.05$).

Table III-3

11 x 8-1/2

Table III-3. ^{14}C-Chlorobenzene Metabolite Profiles in Rat Urines (Sullivan, et al., 1983)

Exposure concentration (ppm)	Exposure regimen[b]	Percentage of ^{14}C-metabolites recovered[a]				
		Initial	G/S	Mercap	Unextracted	Recovered %[c]
100	S	0.8 ± 0.1	27.5 ± 1.1	69.7 ± 0.6	2.0 ± 0.3	98.7 ± 3.8
100	M	1.0 ± 0.0	29.4 ± 1.7	67.8 ± 1.9	1.8 ± 0.2	94.7 ± 3.7
400	S	1.5 ± 0.6	30.6 ± 0.8	61.0 ± 0.2[d]	6.9 ± 0.5	89.3 ± 1.5
400	M	1.1 ± 0.2	38.6 ± 3.3	54.8 ± 3.5[d]	5.6 ± 0.6	89.7 ± 0.9
400[e]	S	0.7 ± 0.1	34.1 ± 0.8	59.4 ± 0.8[d]	5.8 ± 0.1	86.2 ± 0.9
700	S	1.7 ± 0.5	41.8 ± 0.8	51.0 ± 0.9[d,f]	5.6 ± 0.5	93.7 ± 3.5
700	M	1.1 ± 0.4	42.5 ± 1.0	51.9 ± 0.9[d]	4.7 ± 0.6	92.3 ± 2.6

[a] The metabolites were separated into four categories: the Initial category contained metabolites extracted into ether from raw, untreated urine; the G/S category contained residues which had been excreted as glucuronide or sulfate conjugates; Mercap was the mercapturic acid category; and Unextracted included all metabolites not extracted from the urine. The values shown are the means and standard errors for n = 3.

[b] S = single exposure, M = multiple exposure.

[c] Recovered % is the percentage of the expected activity, based on liquid scintillation counting of aliquots of raw urine, which was recovered in the metabolite profile analysis.

[d] Significantly different from 100 ppm ($p < 0.05$).

[e] Data from the repeat 400-ppm exposure block, $n' = 6$.

[f] Significantly different from 400 ppm ($p < 0.05$).

exposure and 0% remained 48 hours post-exposure. Plotting of these data revealed a biphasic pattern of elimination, with a fast phase up to 4 hours post-exposure and a slow phase over the remaining periods (sampled at 12, 24, 36 and 48 hours post-exposure) (see Table III-4). The exposure regimen effect was significant only at the 700 ppm concentration, with the multiple-exposure rats showing a shorter fast phase $t_{1/2}$ than single-exposure rats. At this concentration, also, the fast phase increased in length while the short phase decreased in length for animals exposed only once or over the five days. This blurring of the distinction between the fast and slow phases indicates saturation of the metabolic clearance pathways.

In the study by Smith, et al. (1972) discussed under Metabolism, radiolabel collected during 7 days of the study was found in urine as 19.6% of the dose, feces as 1.05% and tissues as 0.05%. Radiolabel in expired air was not measured, but Williams (1959) found 27% of an 0.5 g/kg oral dose given to rabbits was recovered in expired air over a two-day period.

Table III-4

11 x 8-1/2

Table III-4. Apparent Half-lives of Respiratory Elimination Following Inhalation Exposure of Rats to ^{14}C-Chlorobenzene

(Sullivan, et al., 1983)

Exposure concentration (ppm)	Exposure regimen[a]	Apparent $t_{1.2}$ (hr)[b]		r^2
		Fast phase	Slow phase	
100	S	0.81	8.77	0.96
100	M	0.60	9.24	0.99
400	S	1.13	7.62	0.99
400	M	0.83	8.56	0.99
400[c]	S	1.19	7.70	0.98
700	S	3.65[d]	5.73[d]	0.99
700	M	1.62[d,e]	6.60[d,e]	0.99

[a] S = single exposure, M = multiple exposure.

[b] Half-lives ($t_{1.2}$) were calculated from the rate constants of a biphasic log-linear regression.

[c] Data from the repeat 400 ppm exposure block.

[d] Significantly different from the 100- and 400-ppm groups ($p < 0.05$).

[e] Significantly different from single exposure ($p < 0.05$).

IV. SOURCES OF HUMAN EXPOSURE

This chapter summarizes the available data on usage, environmental fate, and occurrence to characterize the potential for exposure to chlorobenzene. A more extensive discussion of the available information on the chlorobenzene in the environment is presented in "Occurrence of Synthetic Organic Chemicals in Drinking Water, Food and Air" (USEPA, 1987).

Humans may be exposed to chlorobenzene from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. Given the available information on chlorobenzene, this analysis of human exposure is limited to drinking water, food, and ambient air. Some individuals will be exposed to chlorobenzene from their occupational environment. These exposures are not evaluated in this document. Intake of chlorobenzene will be affected by age, weight, and lifestyle.

A. USE/ENVIRONMENTAL FATE

The production of chlorobenzene in the United States in 1984 totaled 256 million pounds (USITC, 1985). Chlorobenzene is used as an intermediate in the production of chlorinated pesticides and as a solvent in metal cleaning operations. In 1978, a total of 79,500 kkg were estimated to be released to the atmosphere, with 78,900 kkg (99 percent) attributable to the aforementioned uses (Systems Applications, 1982). Other releases of chlorobenzene to the environment occur from Publicly Owned Treatment Works (POTWs) and from water chlorination treatment processes used for surface waters and sewage effluents (USEPA, 1986a).

Once released into the environment, chlorobenzene binds with soil and is classified as moderately resistant to moderately susceptible to leaching from the soil column (Helling and Turner, 1968). Biodegradation is not believed to be a major factor in removing chlorobenzene (half-life of <21 weeks) from soils (Wilson et al., 1984). However, available information concerning this subject is limited. Volatilization is believed to be an important removal process for chlorobenzene in contact with air. Volatilization of chlorobenzene from beneath the soil surface would be much slower, decreasing rapidly with increasing soil depth (Lyman et al., 1982). Biodegradation, hydrolysis, and adsorption to sediments are not believed to be important removal processes for the compound in natural waters (Callahan et al., 1979; USGS, 1983).

Chlorobenzene released to surface water is expected to be removed by volatilization. Depending on exact conditions, the half-life of chlorobenzene in surface water is expected to range from 0.77 hours to 53 days (USEPA, 1987).

B. OCCURRENCE

Drinking Water

Several national surveys, including the NSP, the 1978 CWSS, and the GWSS, as well as many surveys provide information on the occurrence of chlorobenzene in public drinking water supplies. According to available information, chlorobenzene occurs more often and at higher levels in ground water than surface water. This is consistent with the environmental fate data, which indicate

that chlorobenzene will volatilize from surface waters. Based on the national surveys, chlorobenzene is not anticipated to occur at levels greater than 1-5 ug/l in groundwater and less than 1 ug/l in surface waters. Some higher levels of chlorobenzene have been reported to occur in State water quality surveys (USEPA, 1987).

Diet

There are insufficient data to determine the degree of dietary intake of chlorobenzene. The only available information on chlorobenzene levels in food in the U.S. was data on concentrations found in mother's milk. A maximum level of 10,000 ug/l was reported by Erickson et al. (1980).

Air

According to Brodzinsky and Singh (1982) who have compiled available published and unpublished atmospheric data on chlorobenzene, the median concentration in urban/suburban areas was calculated at 1.5 ug/m^3.

C. EXPOSURE SUMMARY

The following table summarizes the exposure levels of chlorobenzene in drinking water, food and air. The drinking water and ambient air exposure levels are presented in a range to reflect the uncertainty arising from the limited amount of

Source	Range of Reported Exposure Levels (Low-High)	Estimated Adult Intake
Drinking Water	0-5 ug/l	0-10 ug/day
Food	--	--
Air	0-1 ug/l	0-20 ug/day

monitoring data available. The upper bound should not be regarded as the maximum possible exposure level. The value was generated based on the highest reported concentration on that media. The estimated daily intake levels are based on the assumption of 2 l/day of drinking water and 20 m^3/day or air.

The available data on exposure to chlorobenzene are quite limited. However, it may be concluded that exposures from drinking water are expected to be small. Also, the compound is expected to occur at low levels and only in a small number of groundwater samples.

Non-drinking water exposures to chlorobenzene are likely to be larger than those from drinking water, but the currently available data are insufficient to make a quantitative estimate. Chlorobenzene occurs in urban and suburban air at levels equivalent to an intake of 20 ug/day. As demonstrated by the TEAM survey (USEPA, 1986b), indoor air exposure for many chemicals may be higher than that from ambient air. Therefore, actual exposure to chlorobenzene may be higher than the available data suggest. There are no data available on the levels of chlorobenzene in food and the contribution from this source cannot be evaluated.

V. HEALTH EFFECTS IN ANIMALS

Chlorobenzene produces sedation and anesthesia after acute oral or parenteral administration. Acute poisoning is characterized by signs of disturbance of the central nervous system (CNS). There may be hyperexcitability, restlessness, muscle spasms or tremors followed by varying degrees of CNS depression. The most frequent cause of death is respiratory failure. Acute exposure at very high levels also may result in liver and kidney damage as well as effects on porphyrin synthesis.

Several investigators have determined acute LD_{50}s and LC_{50}s after oral and inhalation exposure to MCB in several species. These data are presented in Table V-1.

Table V-1. Acute Toxicity Data for Chlorobenzene

Animal	Route	LD_{50}	LC_{50}	Reference
Rat	Oral	2.91 g/kg		Irish, 1963
	Oral	2.39 g/l		Varshavskaya, 1967
Rat	Oral	3.4 g/kg		Vecerek, et al., 1976
Rabbit	Oral	2.83 g/kg		Irish, 1963
	Oral	2.25 g/l		Varshavskaya, 1967
Guinea pig	Oral	5.06 g/l		Varshavskaya, 1967
Mouse	Oral	1.44 g/l		Varshavskaya, 1967
Mouse	Inhalation (2 hours)		20 mg/l	Rozenbaum, et al., 1947
	Inhalation		8.2-9.1 mg/l	Bonnet, et al., 1979
Guinea pig	Inhalation		0.05 mg/l	Lecca-Radu, 1959

In rats, the subcutaneous injection of a 4 to 5 g/kg body weight dose of chlorobenzene caused no immediate effects, but resulted in death in a few days. On autopsy, the rats were found to have necrosis in the liver and kidneys. Larger doses produced CNS depression and death in a relatively short period of time. Between 7 and 8 g/kg were fatal in a few hours (cited in von Oettingen, 1955).

Rozenbaum, et al. (1947) conducted a study of the acute toxicity of CB. In mice receiving 5 to 30 mg/l CB by inhalation over a two-hour period, 65% died, with the onset of narcosis being earlier and the intensity stronger than for comparable doses of benzene. All animals exposed to an atmosphere of 20 mg/l or higher exhibited clonic and tonic spasms and died of respiratory failure. Six rabbits received a series of 2 to 20 doses of 0.09 mg/kg CB over a two-week period. One animal died after six injections; two died after eight injections. Blood dyscrasias consisted of an increase in the number of stabnuclear forms and pseudoeosinophils, while no regular reduction in the number of leukocytes was detected. Kidneys showed a swelling of the tubular and glomerular epithelia. The liver exhibited swelling and deposition of fatty droplets.

In addition to producing hepatic and renal toxicity, chlorobenzene also alters pancreatic function (Yang, et al., 1979). A single intraperitoneal dose of chlorobenzene in sesame oil (5 mmol/kg; 560 mg/kg) was administered to adult male Holtzman rats. Control animals received an equivalent volume of sesame oil (1 ml/kg). Experimental measurements were made 24 hours

later. Bile duct-pancreatic flow (BDPF) was increased 10-fold over that observed in control animals ($p < 0.05$). Protein concentration of the effluent was reduced by more than two-thirds, from 21.7 mg/ml to 6.2 mg/l ($p < 0.05$). The rate of bile flow also was increases significantly ($p < 0.05$). Bile chloride levels and serum GPT activity were not altered. The mechanism by which these changes occur is not known, but it appears not to involve secretin or cholinergic stimulation of the pancreas, nor does it occur secondarily to liver damage. The significance of these findings remains unknown.

Ariyoshi, et al. (1975a) administered 250 mg/kg CB orally to 6 rats for 3 days. Animals were sacrificed 24 hours after the final dose. An increase in △-aminolevulinic acid (△-ALA) synthetase in liver homogenates was observed when compared with controls.

Rimington and Ziegler (1963) gavaged rats with each of seven chlorinated benzenes, including CB. The chemical (in concentrations of 100 to 200 mg/l) was suspended in either 1 percent cellofas, liquid paraffin or propylene glycol. Doses were increased gradually to the point of causation of high porphyrin excretion, but minimum fatality. When 3 rats were given 1,140 mg/kg/day CB for 5 days, increases in urinary coproporphyrin III, uroporphyrin, porphobilinogen (PBG) and △-ALA were observed when compared with controls. Severe liver damage with extensive necrosis and fatty changes occurred. There was also an increase in protoporphyrin and uroporphyrin levels in the liver. When compared with the higher substituted chlorinated benzenes, CB was less potent, requiring a higher dose to effect a similar response.

Rimington and Ziegler (1963) showed that, in rats, long-term oral administration of 500 to 800 mg/kg body weight of chlorinated benzenes (except pentachlorobenzene) led to a hepatic porphyria characterized by elevated levels of precursor porphyrins in liver and feces. The first signs of intoxication included an increase in urinary coproporphyrin and porphobilinogen (PBG). Increased aminolevulinic acid excretion was a late effect. The most active compounds were p-dichlorobenzene, 1,2,4-trichlorobenzene, and 1,2,3,4-tetrachlorobenzene. Histologic examination of the animals' livers showed necrosis and fatty changes over large areas. Mono-, 1,2-di- and 1,2,4-trichlorobenzene were the most toxic.

Cameron, et al. (1937) concluded that chlorobenzene, given subcutaneously or intravenously to an unspecified number of rabbits, caused leukopenia. Three successive daily doses of 1 cc (1,130 mg) caused the white cell count to fall from 7,500 cells/mm^3 to 1,850 cells/mm^3 within a week in a single animal with a gradual return to normal. In contrast, Rozenbaum, et al. (1947) reported an increase in red and white cell count after repeated daily subcutaneous administration of chlorobenzene (0.9 mg/kg for up to 20 doses) to rabbits.

Zub (1979) reported leukopenia as well as lymphocytosis and fatty degeneration of the liver in Swiss mice (five males and five females per group) exposed via inhalation to CB 7 hours/day for three weeks at 2.5 mg/l (2,500 mg/m^3) or for three months at 0.1 mg/l (100 mg/m^3). Only half of the animals exposed to 2.5 mg/l survived until the end of the experiment. In the survivors, loss of appetite, emaciation and marked somnolence

were observed, along with body weight loss. Fatty degeneration was observed in the liver leading to yellow atrophy. White blood cell counts fell significantly in 4 of the 5 survivors. One mouse exhibited leucocytosis which the author attributed to a "neoplastic tendency." The percentage increase in lymphocyte count was offset by a corresponding decrease in the percentage of neutrophils. Animals exposed for three months at 0.1 mg/l averaged white cell counts of 5,210 cells/mm^3 as compared with controls averaging 7,670 cells/mm^3 ($p < 0.001$). Control blood smears showed 60% lymphocytes, while treated smears showed 80%. Neutrophils were exactly the reverse: 39% in the controls and 20% in the treated. Basophils numbered 0.8% in the treated group.

Khanin (1969) exposed rats to 0.1 or 1.0 mg/m^3 continuously for 70 to 82 days. CNS depression was observed as well as toxic encephalopathy and inflammation of internal organs. Histologic foci of large cell hyperplasia in the kidneys and protein dystrophy of the liver also were observed.

Knapp, et al. (1971) reported the results of subchronic toxicity studies with CB in beagle dogs and rats. These studies were conducted by Hazelton Laboratories for the Monsanto Company (Hazelton, 1967a,b). Daily oral doses of 0, 27.25, 54.5 or 272.5 mg/kg/day in gelatin capsules were given to dogs of both sexes five days per week over a 93-day period. there were four males and four females in each dose group. After 14 to 21 doses of 272.5 mg/kg, four of the eight dogs died (2 males and 2 females). these animals showed immature leukocytes, lowered blood sugar

levels and elevated serum glutamic pyruvic transaminase and alkaline phosphatase activities. In some dogs, an increase occurred in total bilirubin and total cholesterol. The elevated enzyme levels, bilirubin and cholesterol, reflect the liver damage that was confirmed upon microscopic examination. The presence of immature leukocytes indicates that chlorobenzene affected the bone marrow at the high dose. Also, at the high dose, gross and microscopic pathology was evident in the kidney, liver, gastrointestinal mucosa and hematopoietic tissue of the dogs that died and, to a lesser extent, in the dogs sacrificed after 65 or 66 daily doses. Signs of toxicity were reported as not consistent in dogs receiving the intermediate dose. One female had four episodes of diarrhea and one of vomiting. One male showed conjunctivitis throughout the study and had one episode of emesis. Three dogs gained weight, three lost weight and two maintained their weights. As a group, there was no statistically significant change from control. One male had an increased kidney:body weight ratio; one female had a slightly increased liver:body weight ratio. No statistically significant changes occurred in the group as a whole. No adverse effects were apparent in the low-dose group.

Knapp et al. (1971) stated that no consistent changes of CB effect were evident in dogs at the intermediate and low levels. However, in Hazelton (1967a), the intermediate dose was described as causing slight and somewhat inconsistent histopathologic changes in liver. The pathology report in Hazelton (1967a) is interpreted to conclude that liver pathology in dogs given

intermediate and high doses (but not the low dose) of CB was probably related to treatment. Hence, the NOAEL is considered to be 27.25 mg/kg/day.

Rats were given 0, 12.5, 50, 100 or 250 mg/kg CB in corn oil by gavage for 93 to 99 consecutive days (Hazelton, 1967b). Male rats showed retarded growth at the highest dose level. At 100 and 250 mg/kg doses a statistically significant increase in liver and kidney weights (absolute and relative to body) was observed in females. No significant histopathological changes were noted. No adverse effects were noted in the lower-dose groups.

Rats, rabbits and guinea pigs were exposed by inhalation seven hours a day, five days a week, for a total of 32 exposures over a period of 44 days to concentrations of 200, 475 or 1,000 ppm (Irish, 1963). the response of the animals in the high-dose group was characterized by "histopathological changes" in the lungs, kidneys and liver. In the middle-dose group, there was an increase in liver weight "histopathology." In the low-dose group, no adverse effects were observed. In none of the groups was a hematological change seen (parameters studied were not identified).

CB was administered orally to rats five days a week for a total of 137 doses over 192 days, in dose groups of 14.4, 144 or 288 mg/kg (Irish, 1963). In the middle- and high-dose groups, there were significant increases in liver and kidney weight and some "histopathological changes" in the liver. No significant changes were observed in the low-dose group. Blood and bone marrow were normal in all animals.

Industrial Bio-Test (IBT) conducted a 90-day inhalation toxicity study for Monsanto using 4 groups of beagle dogs (8 dogs per group: 4 males, 4 females), and 4 groups of albino rats (30 rats per group: 15 males, 15 females) (IBT, 1979). Animals of both species were exposed to 0, 0.75, 1.50 or 2.00 mg/L air (0, 104 ppm, 205 ppm or 273 ppm) 6 hours per day, 5 days per week, for a total of 62 exposures (12.4 weeks). It is unknown if this study has been validated. Five of the eight dogs in the high-dose group and two of the eight dogs in the intermediate-dose group were sacrificed before the conclusion of the test period (on the average, 30th day) because they were in a moribund condition. Weight gains for the high-dose dogs were significantly lower than for the corresponding controls. These animals had lower total leukocyte counts than the controls and elevated values of serum alkaline phosphatase, glutamine pyruvic transaminase and glutamate oxaloacetate transaminase. The high-dose females showed dogs significantly higher absolute liver weights; the mid-dose males exhibited significantly lower absolute heart weights ($p < 0.05$). Both mid- and high-dose females had significantly higher absolute organ weights for the pancreas ($p < 0.05$). Gross and microscopic histologic changes for the seven dogs in moribund condition included enlarged and hardened livers and, in the high-dose animals, vacuolization of the hepatocytes. The bone marrow of 2 males and 3 females in the high-dose group was aplastic; the fourth female in this group exhibited marrow which was hypoplastic. Also, in this high-dose group, 1 male and 3 females showed kidney pathology characterized by cytoplasmic

vacuolization of the epithelium of the collecting tubules. Two males had bilateral atrophy of the seminiferous epithelium of the testes. Weight gains for the intermediate-dose dogs were slightly less than controls. No gross or microscopic abnormalities were observed in any of the surviving intermediate-dose dogs or in any of the low-dose dogs.

Among the rats, no gross or microscopic pathological changes were reported in any of the low- and intermediate-dose rats. Significantly higher organ-to-body weight ratios for liver and kidneys and higher absolute overy weights were observed in the high-dose females. High-dose males exhibited significantly higher absolute kidney weights. Low- and mid-dose males had higher absolute weight as well as organ-to-body and organ:brain weight ratio for the lung than controls and lower results for each parameter in the pancreas.

A 6-month inhalation study in male and female beagle dogs was conducted by Monsanto (1980). the parameters of this study were essentially the same as the aforementioned subchronic study by IBT. Adolescent dogs of both sexes (6 dogs/sex/group) were subjected to 123 6-hour exposures over 6 months at target levels of 0, 0.78, 1.57 or 2.08 mg/l. Actual levels were within ± 10% of the target levels.

Each animal was weighed and examined weekly. Following the last exposure, all animals were examined, sacrificed and necropsied. Over 20 tissues were excised, fixed and prepared for histological examination. The adrenals, brain, heart, kidney, liver, pituitary and testes were weighed. Clinical chemistry evaluation of blood

and urine specimens was done twice before the experiment began, twice during the first four weeks of exposure, once a month over the next four months and at sacrifice. Bone marrow samples aspirated from the pelvic ileum were examined from all animals before exposure and at sacrifice. Marrow was examined from 2 dogs/sex/group at monthly intervals during the experiment. Determination of the myeloid:erythroid precursor cell ratio was made at each time point.

No statistical differences in mean weekly body weights or food consumption between controls and treated groups were observed during the course of the entire study. Emesis, abnormal stools and eye irritation were observed during the course of the experiment, beginning at week 7. No statistical association between exposure and eye irritation was observed. There was a dose-related, sex-independent increased occurrence of emesis, both in terms of the number of animals affected and the persistence of the sign. Treated animals exhibited this sign during weeks 7 to 10 and then again during weeks 17 to 27, while controls exhibited emesis sparingly during weeks 18 to 24. A significant increase in the frequency of abnormal stools was observed among treated females during weeks 7 to 22 ($p < 0.001$).

There was a statistically significant reduction in the absolute adrenal weights of males in the middle and high dose groups ($p < 0.05$). A similar dose-trend relationship, though not statistically significant, was observed in the females. there was no statistically significant interpretative abnormality detected in bone marrow at any dose in either sex.

The investigating laboratory determined that "the various enzyme changes considered to be reflective of liver abnormalities occurred with no consistency that could be linked to individual dogs showing marked emesis and abnormal stools. Sex-independent increases ($p < 0.01$) of alkaline phosphatase were mild and not outside of the normal range for beagles. There were no macroscopic or microscopic lesions evident in dogs that could be attributed to the treatment regimen. Therefore, "no toxicological significance can be demonstrated for the observed statistically significant ($p < 0.05$) increases of liver-to-body weight ratio on middle- and high-exposure group female dogs."

Monsanto concluded that, except for the clinical observations of emesis and abnormal stools and the sporadic, statistically significant increases in alkaline phosphatase, there were no dose-related effects observed at any dose level. A more conservative interpretation of the data would lead to the conclusion that only the lowest dose was without significant effect.

Dilley (1977), in a study performed for NIOSH, exposed groups of 32 male rats and 32 male rabbits to 0, 75 or 250 ppm chlorobenzene vapor 7 hours/day, 5 days/week for up to 120 exposures (24 weeks). At 5, 11 and 24 weeks of treatment, groups of animals were sacrificed. Hematological, clinical, chemical, gross and microscopic histological changes were examined.

For the rats, the data showed that, at 11 weeks, there were increased absolute liver and heart weights and increased organ:body weight ratios for liver in the low dose rats. This group also showed increased liver:brain, heart:brain and kidney:brain ratios.

The high-dose rats exhibited only increased liver:body weight ratio. In animals from both dose groups, there were occasional focal lesions in the adrenal glands, focal lesions in the kidney tubules and liver and kidney congestion which appeared to be treatment related. No chemical-related pathology was evident at 24 weeks.

At 11 weeks, in the rats at both doses, there was an increase in the number of blood platelets and reticulocytes and a decrease in microcellular volume with an increase in microhematocrit. In the high-dose group, the white cell count was increased along with the percentage of polymorphonuclear leukocytes. At 24 weeks, the only significant difference seen was an increase in the percentage of reticulocytes in the high-dose group ($p < 0.01$).

SGOT activity was decreased in the high-dose rats at all three sacrifice times.

In the rabbits, dose-related renal congestion was apparent at 5 weeks, some renal congestion at 11 weeks, but no treatment-related changes were seen at 24 weeks. In addition, increased liver and lung weights were noted in the high-dose group at 24 weeks. While the white blood cell count was significantly increased at 5 weeks in the high-dose animals, this difference was not observed at the 11 and 24 week sacrifice. However, at 24 weeks, the numbers of polymorphonuclear leukocytes were increased significantly ($p < 0.05$). SGOT activity was decreased significantly in the high dose animals at all sacrifice times. Other significant changes seen at 5 weeks in one or both dose groups did not persist after 24 weeks.

Varshavskaya (1968) studied the chronic effects of CB on white rats. The rats were treated daily with solutions of CB in oral doses of 0.1, 0.01 or 0.001 mg/kg. Controls received the vehicle, sunflower oil, alone. The experiments lasted nine months. At the 0.1 mg/kg dose, effects (not specified) of the greatest magnitude on the nervous system were observed. Lesser effects were observed at 0.01 mg/kg and no adverse effects were seen at 0.001 mg/kg. CB, at 0.1 mg/kg, caused a statistically-significant inhibition of erythropoiesis, thrombocytosis, inhibition of mitotic activity in the bone marrow and eosinophilia. Similar but less pronounced effects were observed at 0.01 mg/kg, whereas 0.001 mg/kg had no effect. At doses of 0.1 and 0.01 mg/kg, MCB caused a significant increase in blood alkaline phosphatase and blood serum transaminase activity, decreased the number of sulfhydryl groups in whole blood, markedly decreased alkaline phosphatase, but moderately increased acid phosphatase in the liver and kidneys. The liver and kidneys showed decreased concentrations DPN, TPN, succinic dehydrogenase, glucose-6-phosphatase and alpha-glycerophosphate. At 0.001 mg/kg, there were no measurable changes. The results of the studies described above along with other experiments are summarized in Table V-2.

Carcinogenicity

Chlorobenzene has undergone study in the NTP Bioassay program to determine its carcinogenic potential. All studies have been completed. A final report of the 104-week chronic study is now available. All studies were conducted by Battelle's Columbus Laboratories with Fischer 344 rats and B6C3F$_1$ mice (NTP, 1985; Battelle's Columbus Laboratories, 1977a,b, 1978a,b,c,d).

Table V-2

11 x 8-1/2

Table V-2. Chlorobenzene Toxicity Studies

(Subacute Exposure)

Species	Dose and Duration	Effect	Reference
Rats	1,140 mg/kg/day for 5 days (oral)	Increased urinary coproporphyrin porphobilinogen, delta-aminolevulinic acid; fatty and necrotic liver damage	Rimington & Zeigler, 1963
Rats	1 cc/day, 3 days (subcu. or i.v.)	Leukopenia	Cameron, et al., 1937
Rabbits	0.9 mg/kg/injection up to 20 injections (subcu.)	Swelling of convoluted tubule epithelium and glomeruli in kidneys, cellular swelling and fat deposition in liver	Rozenbaum, 1947
Mice	2.5 mg/l, 7 hours/ day for 3 weeks (inhalation)	Leukopenia, lymphocytosis, fatty degeneration in liver	Zub, 1979

Table V-2. Chlorobenzene Toxicity Studies (continued)

(Oral* Subchronic)

Species	Dose and Duration	Effect	Reference
Rats	0.1, 0.01+, 0.01 mg/kg/day for 9 mo.	Statistically significant inhibition of erythropoiesis, thrombocytosis and mitotic activity in marrow; inhibited higher cerebral cortex activity, neutropenia	Varshavaskaya, 1967
Dogs	27.25, 54.5+ or 272.5 mg/kg/day 5 days/week for 13 weeks	Diarrhea, vomiting, conjunctivitis. Cachexia; icterus; hepatic and renal discoloration; distended gall bladder; increased organ/body weight ratios for internal organs; death; increased numbers of monocytes; decreased blood sugar level and number of leukocytes. Immature leukocytes, low blood sugar, elevated SGPT and alkaline phosphatase, increased total bilirubin and total cholesterol, gross and/or microscopic pathology of liver and kidney	Knapp, et al., 1971; Hazelton, 1967a
Rats	12.5, 50, 100+ 250 mg/kg/day for 93 to 99 days	Retarded growth of male rats (high level). Liver and kidney weights significantly increased.	Knapp, et al., 1971; Hazelton, 1967b

* Administered by gavage or capsule.
+ Lowest adverse effect dose.

Table V-2. Chlorobenzene Toxicity Studies (continued)

(Subchronic Inhalation)

Species	Dose and Duration	Effect	Reference
Rats	0.1 or 1.0 ug/l for 70-82 days, continuously	Encephalopathy, inflammation of internal organs, protein dystrophy in liver, regenerated cells in liver, focal giant-cell hyperplasia in kidneys	Khanin, 1969
Rats	1.0 ug/l, for 60 days, continuously	Distorted chronaxie of antagonist muscles, increased blood cholinesterase activity, lowered alpha-globulin content of blood serum	Tarkhova, 1965
Rats	0.1 mg/l, 7-14 weeks	Chronaximetric inhibition preceding enzymatic changes	Pislaru, 1960
Rats	0.1 mg/l for 3 hrs., alternate days for 37 weeks	Temporary inhibition of extensor tibialis chronaxie after 7-14 weeks	Gabor and Raucher, 1960

Table V-2. Chlorobenzene Toxicity Studies (continued)

(Subchronic Inhalation)

Species	Dose and Duration	Effect	Reference
Dogs	0, 0.75, 1.50 or 2.00+ mg/l, 6 hr/day, 5 days/week, 13 weeks	Enlarged and hardened liver, vacuolated hepatocytes, cytoplasmic vacuolation of renal collecting tubules, bilateral atrophy of seminiferous tubules, lower total leukocyte counts, elevated serum AP, GOT, GPT	Industrial Biotest for Monsanto, (IBT, 1979)
Dogs	0, 0.78, 1.57+ or 2.08 mg/l, 6 hr/day, 5 days/week for 6 months	Increase in emesis and abnormal stools; intermittent increases in blood alkaline phosphatase; statistically-significant decrease in adrenal weights and increase in liver/body weight ratio at two highest doses.	Monsanto, 1980
Rats and Rabbits	0, 75 or 250 ppm (0.075 or 0.25 mg/l) 7 hr/day, 5 days/ week, up to 120 exposure days	Trend toward microcytic anemia, decrease in SGOT activity, focal lesions of adrenal cortex, tubular lesions in kidney, congestion in liver and kidneys in rats	Dilley, 1977
Mice	0.1 mg/l, 7 hrs/day, 3 months	Leukopenia, lymphocytosis	Zub, 1979

+ Lowest adverse effect dose reported.

In the acute toxicity study, rats and mice (five animals of each sex/species/dose level) were gavaged with single doses of 0, 250, 500, 1,000, 2,000 or 4,000 mg/kg CB in corn oil and observed for up to 14 days (NTP, 1985; Battelle, 1977a,b). At the end of the 2-week observation period, survivors were sacrificed without necropsy or histopathological examination. No clinical chemistry studies were done. On the second day, 3 of 5 male and 4 of 5 female rats receiving 4,000 mg/kg died. One male rat in the 500 mg/kg group and one in the 2,000 mg/kg group died on day 2. Two females in the 1,000 mg/kg group died on day 3. Of the mice, 19 males and 14 females died during the course of the study. All males from the three highest dose groups died on day 2 or 3. One in the 500 mg/kg group died by day 2 and three from the lowest dose group died on day 2 or 3. All female mice in the two highest dose groups were dead by day 6. Two of the 1,000 mg/kg-dosed group were dead by day 3 and two others at 250 mg/kg died later. Thus, it would seem that mice were more severely affected by their acute exposure to CB than were rats, with male mice being more susceptible than the females.

Based upon the results of the acute toxicity study, doses were selected for repeated oral dose studies. Mice were gavaged once daily for 15 days with doses of 0, 30, 60, 125, 250 or 500 mg/kg CB in corn oil (5 animals/sex/dose level) (NTP, 1985; Battelle, 1978a). Five males and one female died during the course of the study, but none of the deaths were considered to be treatment-related. No treatment-related effects were noted in the survivors. Rats were gavaged once daily for 15 days with

doses of 0, 125, 250, 500, 1,000 or 2,000 mg/kg CB in corn oil (Battelle, 1978b). A total of 10 males and females died during the course of the 15-day study. All five males and all five females at the highest dose level died by day 4 or 6, respectively. All five animals of each sex at 1,000 mg/kg died by day 15. The only significant finding upon gross inspection was that of pale livers (yellow atrophy), indicating hepatotoxicity. This finding was observed in those rats at the two highest dose levels. The 500 mg/kg dose was judged to be the no-effect level in both species.

Subchronic toxicity studies were conducted in which rats and mice were gavaged five times weekly for 13 weeks with 0, 60, 125, 250, 500 or 750 mg/kg MCB in corn oil (10 animals/species/sex/dose level) (NTP, 1985; Battelle, 1978c,d). Mean initial dose group body weights were: 116-126 g in male rats; 101-110 g in female rats; 24-26 g in male mice; and 19-21 g in female mice. Animals surviving the 91 days of treatment were subjected to full necropsies, weighing of organs and histopathological evaluation. Additional studies performed at the end of the exposure period included urinalysis (only of animals at the highest surviving dose and of controls), hematology and clinical chemistry.

Among the 60 mice, 26 males and 22 females died prior to the scheduled sacrifice. All ten males receiving 500 or 750 mg/kg died within the first week of the study. Six males in the 250 mg/kg dose group died by week 10. All 10 females at 750 mg/kg died by week 9; seven females in the 500 mg/kg group and four in the 250 mg/kg group died during the last three weeks. The rats had a higher survival rate. Fourteen males and 11 females died

before the study ended. No males at 750 mg/kg died early, but four died at the 500 mg/kg level. One control died in week 9. The first male died in week 1, the first female succumbed in week 6.

While average individual weekly food consumption by the mice did not differ among the experimental groups, body weight gain was significantly reduced over the entire course of exposure in certain of the treated groups when compared with controls. The percentage decrease in final body weight relative to control was 20% in males in the 250 mg/kg group and 15% in females given 500 mg/kg.

Among the 60 rats, 25 died early (14 males and 11 females). Deaths included 9 of the 10 males at the 750 mg/kg dose, 4 of the 10 at 500 mg/kg and 1 control. Eight of 10 females receiving 750 mg/kg died along with 3 of 10 at 500 mg/kg.

In contrast to the mice, both male and female rats at the two highest doses showed a decrease in food consumption. Males at 750 mg/kg ate 21% less than controls, males at 500 mg/kg, 9% less. Females at 750 mg/kg ate 2.2% less; females at 500 mg/kg ate 13% less. Decreases in final body weight relative to controls increased as the treatment dose increased, with a 12% difference at 250 mg/kg and 13% at 500 mg/kg (6 survivors) and 750 mg/kg (one survivor) for males compared with the controls. In females, 12% and 19% differences were seen at 500 (7 survivors) and 750 mg/kg (2 survivors), respectively.

No clinically-significant chlorobenzene-related changes were observed in any of the hematological parameters measured in either species. None of the clinical chemistry parameters measured were altered in the mice. However, in the rats, SGPT and gamma-glutamyl

transpeptidase (GGPT) levels were elevated in the two females and one male surviving after treatment with 750 mg/kg. Urinalyses of the controls and survivors from the 500 and 740 mg/kg groups revealed a dose-dependent polyuria with concomitant decreases in specific gravity and creatinine concentration. Also, at the two highest doses, urinary coproporphyrin excretion was increased in both sexes of rats. In mice, the increase was observed only in females at 250 and 500 mg/kg.

Liver:body weight ratios were significantly increased in mice of both sexes at 500 mg/kg and higher. Some increase (not statistically significant) was seen in males at 125 mg/kg. Both male and female rats at 250 and 500 mg/kg showed a significant increase. Mice and rats at the three highest doses all exhibited significant histopathological changes consisting of hepatic necrosis, nephrosis, myeloid depletion, lymphoid depletion and lymphoid necrosis. Minimal histopathological changes were seen in male mice at 125 mg/kg.

Doses for the chronic study were selected on the basis of the findings observed in the subchronic studies. Both sexes of rats and female mice received 60 or 120 mg/kg/day; male mice received 30 or 60 mg/kg/day. Doses were administered by gavage in corn oil, 5 days/week for 103 weeks. Groups of 100 rats and 100 mice (50 of each sex) served as vehicle controls, receiving corn oil only on the same schedule as the treated animals. Other groups of 100 rats and 100 mice served as untreated controls.

The following information is presented in the final NTP technical report on the bioassay for chlorobenzene (NTP, 1985).

Mean body weights of dosed animals were essentially the same or greater than those of the controls throughout the study. Survial of low-dose male rats, all female rats and mice and high-dose male mice was not affected adversely by exposure to chlorobenzene (see Tables V-3 and V-4). The survival rate for the high-dose male rats (26/50, 52%) was significantly lower ($p < 0.014$) than for the vehicle controls (39/50, 78%). The survival rate for low-dose male mice was significantly lower than vehicle controls (28/50 or 56% compared with 39/50 or 78%, respectively).

According to the original histopathological evaluation, there was an apparent increase in the occurrence of hepatocellular necrosis and decreases in the occurrence of hepatocellular basophilic change and granulomatous inflammation in treated rats of both sexes. Review of the slides by a second pathologist determined that there was no significant difference. Both individuals agreed that the necrotic lesions were minimal to mild in severity in both chlorobenzene-treated groups. Therefore, it was concluded that the evidence for mild chlorobenzene-induced hepatocellular necrosis in this study is considered equivocal.

Among the mice, the survival of the low-dose males was significantly lower than that of the controls ($p < 0.031$). Non-neoplastic pathology was unremarkable.

Neoplastic nodules occurred in the NTP (1985) carcinogenicity bioassay in male rats. The incidence of animals with nodules was significantly higher in the high-dose males than in the vehicle control males, by all three statistical tests: life table analysis, incidental tumor test and Cochran-Armitage trend and Fischer exact

Table V-3. Rat Survival Rates at 104 Weeks (NTP, 1985)

Exposure Dose (mg/kg/day)	Males		Females	
	Number	Percentage	Number	Percentage
0 (untreated)	34/50*	68%	37/50	74%
0 (vehicle)	39/50	78%	29/50	58%
60	32/50	64%	30/50	60%
120	26/50	52%†	31/50	62%

* $\frac{\text{Number of survivors}}{\text{Number of animals at start of study}}$

† $p < 0.014$, when compared with vehicle controls

Table V-4. Mouse Survival Rates at 105 Weeks (NTP, 1985)

Exposure Dose (mg/kg/day)	Males		Females	
	Number	Percentage	Number	Percentage
0 (untreated)	35/50*	70%	37/50	74%
0 (vehicle)	39/50	78%	40/50	80%
60	29/50	56%†		
120			38/50	76%

* $\frac{\text{Number of survivors}}{\text{Number of animals at start of study}}$

† $p < 0.031$, when compared with vehicle controls

tests (Table V-5). However, hepatocellular carcinomas were observed only in the vehicle control males, and the combined incidences of nodules and carcinomas were increased only by life table analysis with borderline significance ($p = 0.048$, $p < 0.05$) in the high-dose group. No significant increases in carcinomas or nodules, singly or combined, were seen in females.

Also, in the NTP (1985) study in rats, interstitial tumors of the testis were observed with a positive trend in the life table test, and the incidence in the high-dose males was significantly higher than in the vehicle controls by the life table test, but not by either the incidental tumor or Fischer exact tests. A transitional cell papilloma in the bladder was found in one low-dose male and one high-dose male. Endometrial polyps of the uterus were observed with a significantly lower incidence in the low-dose group than in the control females. A renal tubular cell adenocarcinoma was found in one high-dose female.

No site-specific tumors occurred at statistically significant increased or decreased incidences in treated mice of either sex (NTP, 1985).

The NTP has concluded that the doses used in these chronic studies were adequate for carcinogenicity. In addition, NTP proposed that under the conditions of the test, chlorobenzene administration increased the occurrence of hepatic lesions reported as neoplastic nodules of the liver in the high-dose male rats, providing some but not clear evidence of carcinogenic activity in male rats. Carcinogenic effects of chlorobenzene were not observed in female rats or mice of either sex.

Table V-5. Analysis of Liver Tumors in Male Rats: Statistical Comparisons of Treated to Vehicle Controls (NTP, 1985)

	Untreated Control	Vehicle Control	60 mg/kg	120 mg/kg
Neoplastic Nodule				
Tumor Rates				
Overall	4/50 (8%)	2/50 (4%)	4/49 (8%)	8/49 (16%)
Adjusted	10.4%	4.5%	12.5%	29.3%
Terminal	2/34 (6%)	0/39 (0%)	4/32 (13%)	7/26 (27%)
Statistical Tests				
Life Table		P=0.005	P=0.255	P=0.010
Incidental Tumor Test		P=0.011	P=0.290	P=0.021
Cochran-Armitage Trend Test		P=0.027		
Fisher Exact Test			P=0.329	P=0.043
Carcinomas				
Tumor Rates				
Overall	0/50 (0%)	2/50 (4%)	0/49 (0%)	0/49 (0%)
Adjusted	0.0%	5.1%	0.0%	0.0%
Terminal	0/34 (0%)	2/39 (5%)	0/32 (0%)	0/26 (0%)
Statistical Tests				
Life Table		P=0.139N	P=0.283N	P=0.331N
Incidental Tumor Test		P=0.139N	P=0.283N	P=0.331N
Cochran-Armitage Trend Test		P=0.098N		
Fisher Exact Test			P=0.253N	P=0.253N
Neoplastic Nodule or Carcinoma				
Tumor Rates				
Overall	4/50 (8%)	4/50 (8%)	4/49 (8%)	8/49 (16%)
Adjusted	10.4%	9.4%	12.5%	29.3%
Terminal	2/34 (6%)	2/39 (5%)	4/32 (13%)	7/26 (27%)
Statistical Tests				
Life Table		P=0.033	P=0.532	P=0.048
Incidental Tumor Test		P=0.054	P=0.570	P=0.083
Cochran-Armitage Trend Test		P=0.121		
Fisher Exact Test			P=0.631	P=0.168

Developmental Effects

John, et al. (1984, 1980) reported on the results of the preliminary study conducted to determine the doses to be used in the final study protocol. Groups of pregnant rats and rabbits were exposed via inhalation to 0, 300, 1,000 or 3,000 ppm CB 6 hours/day on days 6-15 of gestation (rats) or days 6-18 of gestation (rabbits). All ten of the rats exposed to 3,000 ppm died or were sacrificed in moribund condition before the exposure period was over. Four of seven rabbits also died. The three survivors showed depressed body weight gain. One of the three rabbit litters contained only implantations undergoing resorption, suggesting embryotoxic effects.

At inhalation exposures of 1,000 ppm, rats and rabbits exhibited decreased food consumption and body weight gain. There was a statistically significant increase in liver:body and kidney:body weight ratios in the rats. Also, in rats, a statistically significant increase in resorption incidence occurred, again indicating embryolethality. Rabbits showed a slight, but not statistically significant, increase in resorptions. At 300 ppm, no evidence of significant toxicity in either dams or embryos was observed.

The full teratology study was conducted in two phases (John, et al., 1984; Hayes, et al., 1982). In Phase I, bred rats and inseminated rabbits were exposed via inhalation to 0, 75, 210 or 590 ppm CB, 6 hours/day during the period of major organogenesis (days 6-15 for rats; days 6-18 for rabbits). Rat groups contained 32 or 33 animals; rabbit groups numbered 30 animals.

Animals were observed daily for overt toxicity and weighed on days 6, 9, 12, 16 and 21 (rats or days 6, 9, 12, 15, 19 and 29 (rabbits). Food and water consumption of rats were recorded every third day beginning on Day 6 of gestation. Maternal liver weights in both species were determined at sacrifice (day 21 for rats; day 29 for rabbits). The following data also were recorded: 1) number and position of fetuses in utero; 2) number of dead and live fetuses; 3) number and position of resorption sites; 4) number or corpora lutea; 5) sex, body weight and crown-rump length of each fetus, and 6) any gross external alterations.

Among the rats, no maternal deaths or overt toxicity occurred in animals of any dose group during the exposure period. Nevertheless, some evidence of maternal toxicity was seen in the high dose group (i.e., decreased body weight gain and increased absolute and relative liver weights). Food consumption also was decreased significantly in this group on days 6 to 8, but was increased significantly on days 15 to 17. this phenomenon was not considered to be toxicologically significant. Water consumption in the high dose group was elevated significantly on days 9 to 17.

No effects were seen in rats on pregnancy rates, mean litter size, incidence of implantations undergoing resorption, fetal sex ratios or fetal body measurements at any dose level. The incidence of major malformations, whether considered collectively or singly, was not increased significantly for any treatment groups when compared with the control group. There were statistically significant decreases in the incidence of focal necrosis in the liver of fetuses from the two higher dose groups and in the incidence of

slight cerebral hemorrhage in the low dose group. The investigators did not consider these changes to be toxicologically significant.

There was an increased incidence in certain minor skeletal variants in the rat fetuses. The incidence of delayed ossification of the centra of the cervical vertebrae was increased significantly in the low- and high-dose groups. Also, there was a statistically significant increase in the occurrence of bilobed centra of the thoracic vertebrae and a decrease in the incidence of spurs in the fifth cervical vertebrae in the high dose group. The investigators concluded that these findings were not toxicologically significant or indicative of a teratogenic response. Two maternal deaths occurred in rabbits during the Phase I study. One control female died on day 18 and one from the low-dose group on day 29. Four females delivered early (2 controls, 1 exposed at 75 ppm and 1 at 210 ppm). Another animal exposed to 210 ppm died of pneumonia during delivery.

There were signs of maternal toxicity in pregnant rabbits exposed to 210 or 590 ppm. Statistically significant increases in absolute and relative liver weights were observed. Significant differences in these parameters were not observed in the low-dose group when compared with the controls. No significant differences were noted with respect to body weight or body weight gain.

Pregnancy rates in the rabbits were not affected in treated animals, nor were mean litter size or incidence of implantations undergoing resorption. Statistically significant increases in the percent preimplantation loss was observed in the low and intermediate dose groups, but since exposure was not initiated

until day 6 of gestation, this phenomenon was considered to be unrelated to exposure to monochlorobenzene.

A statistically significant difference in fetal sex ratio (58% male:42% female) was observed in the litters from the low-dose group. A significant increase in the mean fetal crown-rump length in litters from the intermediate-dose group also was seen. The changes were not considered toxicologically significant. Mean fetal body weights/litter were not altered significantly.

The incidences of external, soft tissue or skeletal alterations among litters from exposed rabbits did not differ significantly from controls. Whether considered singly or collectively, the incidences of major malformations observed in each of the exposed groups were not significantly different from the controls. However, the variety and number of major malformations in the control group occurred at levels slightly higher than the laboratory's historical controls. Thus, it was decided that the rabbit teratology study would be repeated. The study was repeated as Phase II.

The protocol used in Phase II was essentially the same as that used in Phase I, except that the dose levels employed were 0, 10, 30, 75 and 590 ppm.

No maternal deaths occurred during the Phase II study. The only sign of maternal toxicity was a statistically significant increase in absolute liver weights in the high-dose group. The liver:body weight ratio was not altered significantly at this or any other exposure level. No differences in maternal body weights or body weight gain occurred.

Again, statistically significant increases in percent pre-implantation loss were observed, both in the 75 and 590 ppm dose groups. Also, a significant increase in the percentage of implantations undergoing resorptions occurred in the 590 ppm group when compared with the controls (61% of the litter representing 12% of the fetuses in the 590 ppm group vs. 41% of the litters representing 6% of the fetuses in the control group). The investigators did not consider this observation to be toxicologically significant, stating that the 61% was within the normal range for that laboratory. Fetal sex ratios and body measurements were not altered significantly.

The incidences of major malformations in the exposed groups, whether considered collectively or singly, were not significantly different from the incidences observed in the controls. In fact, statistically significant decreases were seen in the 10 and 590 ppm groups.

The investigators concluded that, under the conditions of the studies, inhalation of CB for 6 hours/day at exposures up to 590 ppm during the period of major organogenesis was not teratogenic to rats and rabbits. The sole remarkable fetal effect was slightly delayed skeletal development at 590 ppm, a maternally toxic concentration.

Reproductive Effects

An inhalation two-generation reproduction study in rats by Nair et al. (1987) was designed to evaluate the effects of CB on the reproductive performance and fertility of rats through two

consecutive generations (F_0, F_1). Chlorobenzene was administered as vapor by the inhalation route with animals exposed in $6m^3$ glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 450 ppm; included in the study was a chamber exposed, sham-air control group. Each study group was comprised of 60 CD® rats (30/sex/generation). F_0 adult animals were exposed daily (6 hrs/day) for a 10 week pre-mating treatment period and during mating. Once mated, females were exposed (6 hr/day) during gestation (Days 0-19) and lactation (Days 5-21) of the F_1 litters. F_0 males continued to be treated daily during the post-mating period until sacrifice. Similarly, F_0 females continued to be treated daily post-weaning until sacrifice (soon after weaning of the last litter). F_1 pups (2/sex/litter) selected at Day 21 (weaning) became a pool of animals from which the F_1 adult generation was selected. These animals were exposed at 29 days of age to comparable dose levels as the dams. Once the F_1 adult generation was chosen, these animals received an 11-week pre-mating treatment period. Exposure of animals during the mating, gestation and lactation intervals of the F_2 litters was similar to that for the F_0 animals.

F_0 and F_1 adult males were sacrificed as a group several weeks post-mating (i.e., approximately three-four weeks); F_0 and F_1 females were sacrificed as a group after the last litter (F_1 and F_2, respectively) was weaned. Each adult generation animal was given a gross postmortem examination and liver, kidneys and reproductive tissues saved (10% formalin). Liver, kidney and brain weights were recorded at sacrifice and absolute and relative

(organ/body and organ/brain) liver and kidney weight data were evaluated. Initially, microscopic evaluations were restricted to tissues for the control and high-dose groups, both generations; however, these evaluations were extended to liver, kidneys and testes/epididymides for all low- and mid-dose males (F_0, F_1). F_1 and F_2 pups were given a gross external and internal examination and discarded; only abnormal tissues were saved (10% formalin). F_1 pups were sacrificed either at weaning (Day 21) or at the time of selection for the F_1 adult generation. F_2 pups were sacrificed at Day 21 of lactation.

Mean analytical concentrations (±S.D.) for the F_0/F_1 exposure intervals were similar to targeted exposure levels: low-dose - 51 ± 5/49 ± 4; mid-dose - 151 ± 8/150 ± 11; and high-dose - 451 ± 25/454 ± 21.

No mortality occurred among the control or treated animals in either adult generation.

In the low-dose group, no adverse effect of treatment was evident in the F_0 generation. In the F_1 generation, no adverse effect of treatment at the low-dose level was evident in body weight or food consumption data, physical observations, reproductive performance of F_2 litter data. Similarly, F_1 and F_2 pups nursed and weaned to low-dose females showed no adverse effect of treatment. At sacrifice of the F_1 low-dose males, the mean liver to body weight ratio was statistically significantly higher than control. No adverse effect of treatment at the low-dose level was evident from the gross postmortem evaluations of the microscopic evaluations of livers, kidneys and testes/epididymides. Histopathologic

lesions of the livers of the low-dose F_1 males were not evident, and absolute liver weights and liver to brain weight ratios in F_1 low-dose males were not significantly higher than controls. Therefore, without additional supporting evidence for the increased liver to body weight ratio in low-dose F_1 males as a treatment-related effect, 50 ppm is taken as a NOAEL in this study.

In the mid- and high-dose groups, no adverse effect of treatment was evident in body weight, food consumption, physical observations, reproductive performance or fertility in the adult generations (F_0, F_1). Similarly, no adverse effect of treatment was evident from the evaluation of pups delivered, nursed and weaned to these same females (F_0, F_1).

At sacrifice of the mid- and high-dose adults, mean liver weight data (absolute and relative to brain or body weights) were higher than control and many of these differences from control data were statistically significant particularly for the males (F_0, F_1). Microscopic examination of tissues for the F_0 and F_1 adults revealed hepatocellular hypertrophy and renal degenerative and inflammatory lesions. These lesions were noted with increased incidence and severity in the mid- and high-dose males for both adult generations. These changes were considered to be compound-related. There was also an increase in the incidence of testicular degenerative changes, unilateral or bilateral, in the high-dose males (F_0 and F_1 generation) and in the F_1 mid-dose males.

Mutagenicity

Abnormal mitotic division of the onion, Allium cepa, on treatment with several halogenated benzenes has been described

by Ostergran and Levan (1943). Chlorobenzene at concentrations of 1 x 10^{-3} mol (precipitate in water) caused full c-mitosis disturbances. Its activity was marginal at 3 x 10^{-4} mol (clear aqueous solution) and negative at 1 x 10^{-4} mol.

Prasad and Pramer (1968) and Prasad (1970) investigated the mutagenic potential of chlorobenzene at a concentration of 200 ug/ml. The chemical was evaluated by determining the frequency of back mutation of the methionine-requiring locus in the fungus, Aspergillus nidulans. No metabolic activation was employed. Chlorobenzene was nonmutagenic under these experimental conditions.

Chlorobenzene was nonmutagenic when tested for gene mutation in the Salmonella/microsomal assay (Monsanto, 1976a; Dupont, 1977; Merck, 1978; Simmon, et al., 1979). The compound was tested in Salmonella strains TA 1535, 1537, 1538, 98 and 100 and E. coli WP2 with and without microsomal activation, at concentrations in the medium of 0.01 to 5.0 ul/plate or 150-3,000 ug/plate. Significant cytotoxicity was observed at many of the dose levels in most of the strains. Statistical analyses of the data in these studies did not include numeration of the number of revertants per unit of survivors, an analysis which might provide a better indication of the effect of the compound on the test organisms.

The potential of chlorobenzene to induce specific locus forward mutations was tested in the mouse lymphoma L5178Y cell culture assay system (Monsanto, 1976b). The compound was non-mutagenic at 0.001 ul/ml without enzymatic activation and at 0.001-0.01 ul/ml with activation.

Chlorobenzene was shown to induce reciprocal recombination in the yeast Saccharomyces cerevisiae strain D3 (Simmon, et al., 1979). In the presence of the microsomal activation system, at concentrations of 0.05 and 0.06%, chlorobenzene caused a reproducible increase in the number of recombinants/10^5 suvivors when compared with controls.

At concentrations of 10 or 20 ul/plate, chlorobenzene was equally toxic to the repair-proficient (pol A^+) and repair-deficient (pol A^-) strains of E. coli and to rec^+ and rec^- strains of Bacillus subtilis (Simmon, et al., 1979).

Actions of Combinations

Of the halogenated benzenes, chlorobenzene is the most active in destroying cytochrome P-450. There are some possible interactions between chlorobenzene and cytochrome P-450, but mechanisms remain to be elucidated.

One example of an additive effect with concomitant exposure was reported by Shelton and Weber (1980) in the mouse. A variety of mixtures of carbon tetrachloride and chlorobenzene or carbon tetrachloride and acetaminophen was administered to male albino CD-1 mice. A positive quantal response was considered to be an elevation of plasma alanine aminotransferase activity at least three standard deviations above control. Dose-response curves were developed for each compound separately and the slopes compared with one another. From these data, theoretical dose-response curves for the mixtures were postulated with the prediction that the interaction would be additive in both cases. In the case of the combination of carbon tetrachloride and chlorobenzene, the

observed dose-response curve did not deviate from the curve predicted on the basis of an additive effect ($p < 0.975$). Therefore, the authors classified the carbon tetrachloride:chlorobenzene interaction as concentration additive.

VI. HEALTH EFFECTS IN HUMANS

Most of the incidents reported in the literature are poisonings resulting from inhalation of chlorinated benzenes, including chlorobenzene. Accidental inhalation can occur either at home or at work. There are several cases reported, however, in which CB was accidentally or deliberately ingested (See Table VI-1).

Inhalation exposure to CB causes headaches, irritation of the eyes and upper respiratory tract, sedation and narcosis (Girard, et al., 1969). A two-year-old male survived ingestion of 5-10 cc CB. When hospitalized, he was unconscious, cyanotic and twitching in the head and neck regions (Reich, 1934). No long-term follow-up of this patient was made.

Rozenbaum, et al. (1947) studied 28 workers exposed to variable concentrations of CB vapors. A number of the workers exhibited symptoms that included headache, dizziness, somnolence and dyspeptic disorders. Spastic contractions of finger muscles were observed in nine people; hyperthesia of the hands was found in four people. Two hematological examinations of the workers were conducted. The first revealed normal red cell conditions, but the number of white cells were abnormally low in 11 people. Seven people exhibited thrombocytopenia with two of these individuals suffering from bleeding of the gums and nasal mucosa. Several months later the tests were repeated. The investigators did not state if the workers continued to be exposed during this period. The abnormalities seen in the first examination had returned to nearly normal.

Table VI-1. Human Exposure to Chlorobenzene

(Modified from Ware and West, 1977)

Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
70-year-old female	1958-1964 to a glue containing 70% CB (no benzene or higher homologues)	Headaches, irritation of eyes and upper respiratory tract	Medullar aplasia	1966-"satisfactory" 1977-"mediocre"	Girard, et al., 1969
2-year-old male	Swallowed CB, 5-10 cc	Unconscious, no reaction to skin stimuli, muscle spasms, cyanotic	CB intoxication	Odor of CB present in urine and air exhaled 5-6 days later. General health appeared undisturbed	Reich, 1934
28 workers	Occupational exposure to CB	headache, dizziness, somnulence, dyspeptic disorders	CB intoxication	Abnormal white cell counts in 11 which reverted to normal after termination of exposure	Rozenbaum, et al., 1947
Male worker	Inhalation of CB	General discomfort, massive hemoptysis several hours later	Poisoning due to CB	Not indicated	Ehrlicher, 1963

Gabor, et al. (1962) reported on individuals who were exposed to benzene, CB and vinyl chloride during a three-year observation period. eighty-two workers examined at 1-year time points were found to have an increase in the activity of leukocyte peroxidase at all three sampling times and a slight leukopenia toward the end of the third year.

Ehrlicher (1968) reported that massive hemoptysis occurred in a male worker exposed to a large amount of chlorobenzene sprayed into the atmosphere during an industrial accident. Onset of the bleeding did not occur until several hours after inhalation of the compound. The hemorrhaging was preceded by a feeling of general discomfort. No follow-up studies on this patient were reported.

Girard, et al. (1969) reported the case of the elderly female exposed to a glue containing 70% CB for a period of six years. She presented with symptoms of headache, irritation of the eyes and upper respiratory tract and was diagnosed to have medullary aplasia. Smirnova and Granik (1970) reported on three adults who developed numbness, loss of consciousness, hyperemia of the conjunctiva and the pharynx following exposure to "high" levels of CB. Information concerning the ultimate course of these individuals is not available.

VII. MECHANISMS OF TOXICITY

Many workers have studied the possibility that organ and cellular damage caused by many compounds is mediated via chemically reactive metabolites. Many of the metabolites formed are chemically inert, but certain of the metabolites such as the arene oxides or epoxides may interact with physiological or biochemical processes, causing either pharmacological or toxicological effects. However, it is also possible that there are toxic effects, e.g., narcosis and central nervous system disturbances, due to the parent compound itself.

While the arene oxides of chlorobenzene have not been isolated *in vivo*, evidence suggests that the oxides are formed during the metabolism of the parent compound to 2- and 4-chlorophenol which have been isolated as metabolites (Selander, *et al.*, 1975b). These investigators synthesized the 3- and 4-chlorobenzene oxides. Isomerization of each oxide was examined in the presence of rat liver microsomes at pH 8. Only 4- and 2-chlorophenols (>99.5%) were produced from each oxide, respectively. While it has been shown that 3-chlorophenol also is produced *in vivo* (Smith, *et al.*, 1972) and by the perfused liver (Selander, *et al.*, 1975a), this isomer appears to result from the action of an enzyme which catalyzes the insertion of an oxygen directly into the carbon-hydrogen bond without intervention of an arene bond (Tomaszewski, *et al.*, 1975).

Selander, *et al.* (1975b) performed trapping experiments in which ^{14}C-chlorobenzene was incubated with liver microsomes from

control rats or those pretreated with 3-methylcholanthrene (3-MC) or phenobarbital (PB) in the presence of the 4- or 3-chlorobenzene oxide. Recovery of the two oxides was 47% and 29%, respectively.

Early studies of other halogenated benzenes (HBs) (Brodie, et al., 1971; Reid, et al., 1971; Mitchell, et al., 1971) demonstrated that hepatic necrosis produced on exposure to these compounds results from their conversion to reactive toxic intermediates. The severity of liver necrosis correlated with the extent of covalent binding of metabolites in vitro and in vivo after various pretreatments. These studies also demonstrated that the arene oxide formed is conjugated with glutathione (Reid, et al., 1973; Reid and Krishna, 1973). The glutathione content in the liver was decreased in the presence of large doses of the more active halobenzenes. Conjugation with glutathione was believed to be an important detoxication mechanism. Liver necrosis occurred when glutathione was depleted.

Dalich and Larson (1985) more recently concluded from their work that there was little correlation, in liver, among histopathologic or functional damage, chlorobenzene metabolism, and protein binding. In this study, male Sprague-Dawley rats, 75-125 g, were given single intraperitoneal doses of chlorobenzene in corn oil at doses ranging from 2.0-14.7 mmol/kg (225.2-1,655.2 mg/kg). They found functional evidence of hepatotoxicity in the form of increases in sulfobromophthalene (BSP) retention and increased plasma alanine aminotransferase (ALT) activity early (3 hours) after treatment. Glutathione (GSH) depletion also occurred early after treatment, with the greatest depletion

by eight hours. However, all doses decreased GSH equivalently, i.e., to 31% of control levels, which was accompanied by extensive necrosis. At the lower doses, recovery of GSH levels occurred soon after maximal depletion, but higher doses required longer recovery periods. In contrast to the conclusion by Jollow, et al. (1974), who postulated that reduction of GSH to a threshold level is the critical event preceding covalent binding, Dalich and Larson (1985) proposed that the degree of depletion is not as pivotal as the duration over which GSH is depleted. While histopathologic evidence of liver lesions from chlorobenzene treatment was dose-related over the entire dose range, covalent binding to liver protein was dose-related only through 9.8 mmol/kg. Since cytochrome P-450 levels in liver were decreased to the same extent with all doses, the protein binding did not suggest reduced metabolism due to elimination of cytochrome.

Burka, et al. (1983) studied the monohydroxylation of halobenzenes, including chlorobenzene, by phenobarbital-induced rat liver microsomes. In the case of chlorobenzene, p-chlorophenol was found to be the major metabolite in vivo. The authors found a good correlation of V_{max} with the σ^+ Hammett constant, implying that a positively-charged transition state exists. This suggests that the product-determining step occurs after the rate-determining step. The rate-determining step is postulated to be the formation of a radical cation intermediate.

Just as there are species differences in metabolism, there are differences in the rate and/or extent of metabolism of a halogenated benzene in different organs, i.e., lung may differ

from kidney which differs from liver, etc. Phenobarbital does not increase covalent binding in the lung compared to liver (Reid, et al., 1973; Reid and Krishna, 1973).

Ariyoshi, et al. (1975a,b) have shown an initial increase in content of cytochrome P-450 and an initial decrease in -ALA synthetase activity following administration of chlorinated benzenes. In the time course following a single oral dose of 250 mg/kg body weight, the activity of -ALA synthetase decreased at six hours, was normal at 12 hours and increased at 24 hours. The opposite changes were noted in the content of cytochrome P-450.

In a later publication, the same group of investigators reported a dose-response relationship for the same parameters in a multiple dose study (Ariyoshi, et al., 1981). Animals were injected intraperitoneally with single doses of 50, 100 or 200 mg CB/ml/kg 24 hours before sacrifice. At the lowest dose, no significant increase in -aminolevulinic synthetase activity occurred. However, heme oxygenase activity tripled. Cytochrome P-450 content was not significantly different from control. At 100 and 200 mg/kg, -ALA and heme oxygenase activities were increased further. At 100 mg/kg, cytochrome P-450 levels remained unchanged, but at 200 mg/kg, it was significantly lower than control ($p < 0.005$).

Carlson and Tardiff (1976) studied the effects of chlorobenzene and other halogenated benzenes in adult male rats. All halogenated benzenes except chlorobenzene decreased hexobarbital sleeping time on the same day and/or 14 days following administration of the chemical. In addition, CB induced decreases in cytochrome P-450 content, and increases in glucuronyl transferase levels.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic effects. those chemicals which produce noncarcinogenic effects are believed to have a threshold dose below which no adverse effects occur. Carcinogens are assumed to act without a threshold.

Quantification of Noncarcinogenic Effects

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), formerly called Acceptable Daily Intake (ADI) is calculated. The RfD is defined as a benchmark dose derived from the NOAEL (or LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects, even if exposure occurs over a lifetime. The RfD is calculated as follows:

$$RfD = \frac{\text{(NOAEL or LOAEL)}}{\text{Uncertainty Factor(s)}} = ___ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based on professional judgment, taking the entire data base of toxicological effects for the chemical into consideration. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the Office of Drinking Water (ODW) has modified the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

- An uncertainty factor of 10 is generally used when good acute or chronic human exposure data identifying a NOAEL are available and are supported by good acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is generally used (1) when good acute or chronic toxicity data identifying a NOAEL are available for one or more animal species (and human data are not available), or (2) when good acute or chronic toxicity data identifying a LOAEL in humans are available.
- An uncertainty factor of up to 1,000 is generally used (1) when limited or incomplete acute or chronic toxicity data are available, or (2) when good acute or chronic toxicity data identify a LOAEL, but not a NOAEL for one or more animal species.

The uncertainty factor used for a specific risk assessment is based principally on scientific judgment rather than scientific fact. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor, include the use of a less than lifetime study for deriving a RfD, the significance of the adverse effect and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL is a medium-specific (i.e., drinking water) lifetime exposure, assuming a 100% relative source contribution from that medium, at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL value is intended to provide the health basis for establishing a drinking water

standard ... upon noncarcinogenic health effects. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times (Body\ Weight\ in\ kg)}{Drinking\ Water\ Volume\ in\ L/day} = ___\ mg/l$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect-Level identified from a study of subchronic or greater duration.

Body weight = assumed to be 70 kg for an adult.

Drinking water volume = assumed to be 2 liters per day for an adult.

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (i.e., One-day, Ten-day, and Longer-term) are determined. The HA values are used as an informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using a similar equation as the RfD and DWEL; however, the NOAELs or LOAELs are identified from studies of less than lifetime duration. The HAs are derived as follows:

$$HA = \frac{(NOAEL\ or\ LOAEL) \times (bw)}{(UF) \times (___\ L/day)} = ___\ mg/l$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. One-day HA for a 10-kg child ingesting 1 L water per day, and is generally derived from a study of less than 7 days duration.
2. Ten-day HA for a 10-kg child ingesting 1 L water per day, and is generally derived from a study of less than 30 days duration.
3. Longer-term HA for a 10-kg child ingesting 1 L water per day, and is generally derived from a subchronic study (exposure for 10% of animal's lifetime).

4. Longer-term HA for a 70-kg adult ingesting 2 L water per day, and is generally derived from a subchronic study (exposure for 10% of animal's lifetime).

The One-day HA calculated for a 10-kg child assumes a single acute exposure to the chemical. The Ten-day HA assumes a limited exposure period of 1 to 2 weeks. The Longer-term HA is derived for both the 10-kg child and a 70-kg adult and assumes an exposure period of approximately 7 years (or 10% of an individual's lifetime).

Quantification of Carcinogenic Effects

It is not always simple to decide whether a chemical is a carcinogen or not, since available studies may be limited, inadequate or inconsistent. The EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

- Group A: Known Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal relationship between exposure to the chemical and human cancer.
- Group B: Probable Human Carcinogen. Adequate evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
- Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
- Group D: Not classified. Inadequate evidence of carcinogenicity in animals.

- Group E: No evidence of carcinogenicity. No evidence of carcinogenicity in adequate tests in two different animal species or in both animal and epidemiology studies.

If toxicological evidence leads to the classification of the contaminant as a known or probable human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The bioassay data used in these estimates usually comes from animal studies. In order to predict the risk for humans from animal data, they must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 liters of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure to the chemical via ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may

also be calculated using other models such as the one-hit, Weibull, logit and probit. There is no basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any others. Because each model is based upon differing assumptions, the estimates which are derived for each model can differ by several orders of magnitude.

The scientific database used to calculate and support the setting of risk rate levels has an inherent uncertainty due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose response data usually are available only for high levels of exposure, not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Definition of Acronyms

RfD = Reference dose (mg/kg/day). RfD replaces ADI.

A benchmark dose operationally derived from a NOAEL (or LOAEL) by the consistent application of order(s)

of magnitude uncertainty factors. The RfD is an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects, even if exposure occurs over a lifetime.

$= \frac{\text{NOAEL (or LOAEL)}}{\text{UF(s)}} = ___$ mg/kg bw/day

DWEL = Drinking Water Equivalent Level (mg/L). DWEL replaces AADI.

A medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, non-carcinogenic health effects are not anticipated to occur.

$= \frac{\text{RfD x Bw}}{\text{__ L/day}} = ___$ mg/L (___ ug/L)

where: Bw = 70 kg, assumed body weight of an adult.

__ L/day = 2, assumed water consumption of an adult.

Lifetime HA = Lifetime Health Advisory (mg/L)

The Lifetime HA represents that portion of an individual's total exposure to a contaminant which is attributed to drinking water (not food or air) and would be protective of non-carcinogenic, adverse effects for a lifetime exposure. The percent contribution of the exposure through drinking water (relative sourc contribution) (RSC) is based on actual exposure data or, if the data are not available, the RSC is 20% for organics or 10% for inorganics.

= DWEL x ___% RSC = ___ mg/L

MCLG = Maximum Contaminant Level Goal (mg/L). MCLG replaces RMCL.

The MCLG is a non-enforceable goal which is used to establish the legally enforceable Maximum Contaminant Level. The MCLG is based on health effects, and considers other sources of exposure.

For contaminants not classified as Group A, B or C in EPA's carcinogenicity classification:

MCLG = Lifetime HA.

For Group A and B carcinogens:

MCLG = zero.

For Group C Carcinogens:

$$\text{MCLG} = \frac{\text{Lifetime HA}}{\text{modifying factor (usually 10)}}$$

or

MCLG = range of 10-5 - 10-6 upper confidence limit of cancer risk estimate.

QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories (HAs) are generally determined for exposures of One-day, Ten-days, Longer-term (approximately 7 year exposure) and Lifetime if adequate data are available which identify a sensitive noncarcinogenic end point of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{BW})}{(\text{UF}) \times (___\ \text{L/day})} = ___\ \text{mg/L}\ (___\ \text{ug/L})$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect-Level in mg/kg bw/day.

BW = assumed body weight of protected individual (10 kg for a child and 70 kg for an adult).

UF(s) = uncertainty factor (10, 100 or up to 1000), based on nature and quality of data.

___ L/day = assumed daily water consumption of protected individual (1 L for a child and 2 L for an adult).

Non-Carcinogenic Effects

The principal toxic effects of chlorobenzene (CB) in humans and other animals include central nervous system (CNS) depression, blood dyscrasias, lung, liver and kidney damage. The appearance

and intensity of these and other adverse effects are dependent upon dose and duration of exposure. Deaths in animals following high level acute exposure usually result from the CNS effects (primarily, respiratory failure). No human deaths have been reported following accidental exposure.

The oral LD_{50} for CB in rats and rabbits is about 3 g/kg (Irish, 1963; Vecerek, et al., 1976). Varshavskaya (1967) determined oral LD_{50}s in several species in g/l. Conversion to dose/kg body weight was not possible from the available data. Nonetheless, the relative sensitivity of different species can be inferred (mouse = 1.44 g/l, rabbit = 2.25 g/l, rat = 2.39 g/l, guinea pig = 5.06 g/l). The inhalation LC_{50} in mice was determined by Bonnet, et al. (1979) to be 8.2-9.1 mg/l and by Rozenbaum, et al. (1947) to be 20 mg/l (2-hour exposure).

CB toxicity seen after non-fatal exposures is manifested principally as transient CNS effect (e.g., narcosis, somnolence, muscle spasms), blood dyscrasias (thrombocytopenia, leukopenia, lymphocytosis) and hepatic and renal toxicity. Reports of poisonings in humans described the victims as suffering from headaches, irritation of the eyes and respiratory passages, variable degrees of supression of the level of consciousness, muscle spasms and dizziness (Reich, 1934; Rozenbaum, et al., 1947; Girard, et al., 1969; Smirnova and Granik, 1970). Rozenbaum, et al. (1947) reported transient thrombocytopenia and leukopenia in several exposed workers. They also observed leukocytosis in rabbits receiving a series of 2 to 20 0.9 mg/kg doses of CB over a 2-week period. In contrast, Cameron, et al. (1937) determined

that CB, given subcutaneously or intravenously to rabbits, caused leukopenia when administered in daily doses of 1 ml for three successive days. Zub (1979) reported both phenomena in Swiss mice after inhalation exposure 7 hours/day for three weeks at 2.5 mg/l or for three months at 0.1 mg/l. Mice and rats receiving 250, 500 or 750 mg/kg CB in corn oil by gavage 5 times/week for 91 days exhibited significant histological changes including myeloid and lymphoid depletion and lymphoid necrosis (Battelle, 1978a,b). These effects were not seen at 60 or 125 mg/kg.

Another target organ of CB is the liver, in which the chemical causes necrosis and interferes with porphyrin metabolism, as do other chlorinated benzenes (Rimington and Ziegler, 1963; Varshavskaya, 1967; Khanin, 1969; Knapp, et al., 1971; Zub, 1979). CB also affects the kidney, in which congestive and necrotic changes are seen (Rozenbaum, et al., 1947; Irish, 1963; Khanin, 1969; Dilley, 1977).

Additional effects of CB which have been reported include alteration of pancreatic function (Yang, et al., 1979), pulmonary changes (Irish, 1963; Dilley, 1977) and emesis and abnormal stools (Rozenbaum, 1947).

2. One-day and Ten-day Health Advisories

No experimental data are available from which to derive One-day and Ten-day HAs for either the 10-kg child or the 70-kg adult. The studies studies of rats and mice by the NTP (1985) are not adequate because of inadequate assessment for toxicity in these studies. It is recommended that for this duration of

exposure, the Longer-term HA be applied (see below). Therefore, the One-day and Ten-day Health Advisories are 1.9 mg/l (1,900 ug/l).

2. Longer-Term Health Advisories

Subchronic studies were conducted in which dogs were given chlorobenzene orally by capsule five days per week over 90 days at 0, 27.25, 54.5 or 272.5 mg/kg/day (Knapp et al., 1971; Hazelton, 1967a), rats received 0, 12.5, 50, 100 or 250 mg/kg/day chlorobenzene in corn oil by gavage for 93 to 99 consecutive days (Knapp et al., 1971; Hazelton, 1967b), and both rats and mice were dosed with 0, 60, 125, 250, 500 or 750 mg/kg/day chlorobenzene in corn oil by gavage five times per week over a 13-week period. In the rat and mouse studies, NOAELs of 50 and 60 mg/kg/day were evident largely based on increased liver and kidney weights as well as other adverse effects at higher doses. The 27.25 mg/kg/day dose level is concluded to be a NOAEL in the dog study because of liver histopathology found with higher doses. Because available data support the dog as the most sensitive species of those studied for chlorobenzene toxicity (Knapp et al., 1971 concluded that the dog was more sensitive than the rat to chlorobenzene toxicity in their study), the NOAEL of 27.25 mg/kg/day is selected for calculation of Longer-term HAs. The two generation reproduction study in rats exposed to chlorobenzene by inhalation (Nair et al., 1987) is not considered for Longer-term HAs because exposure was by inhalation and observed treatment-related effects were also found in rats given chlorobenzene orally.

A Longer-term Health Advisory is calculated as follows:

For the 10-kg child:

$$\text{Longer-term HA} = \frac{(27\ \text{mg/kg/day})\ (10\ \text{kg})\ (5)}{(100)\ (1\ \text{L/day})\ \ (7)} = 1.9\ \text{mg/L}\ (1{,}900\ \text{ug/L})$$

where:

27 mg/kg/day* = NOAEL, based upon liver histopathology and other adverse effects at higher doses

10 kg = assumed weight of protected individual

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration)

1 L/day = assumed volume of water ingested by 10-kg child in liters

For the 70-kg adult:

$$\text{Longer-term HA} = \frac{(27\ \text{mg/kg/day})\ (70\ \text{kg})\ (5)}{(100)\ (2\ \text{L/day})\ \ (7)} = 6.8\ \text{mg/L}\ (6{,}800\ \text{ug/L})$$

where:

27 mg/kg/day* = NOAEL, based upon liver histopathology and other adverse effects at higher doses

70 kg = assumed weight of protected individual

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration)

2 L/day = assumed volume of water ingested by 70-kg adult

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*Rounded from 27.25 mg/kg/day.

3. Lifetime Health Advisory

A number of studies utilizing the oral route of exposure are available in which dose-response data are described. These studies identify no-observed-adverse-effect-levels (NOAELs) or lowest-adverse-effect-levels (LOAELs) which might be considered suitable for the derivation of an RfD.

The toxicity of chlorobenzene following oral administration was reported by Dow Chemical Company (Irish, 1963). CB was administered to rats five days/week for a total of 137 doses over 192 days, dose groups of 14.4, 144 or 288 mg/kg. In the middle- and high-dose groups, there were significant increases in liver and kidney weights and some "histopathological changes" in the liver. No significant changes were observed in the low-dose group. Blood and bone marrow were normal in all animals.

The results of this study suggest a NOAEL of 14.4 mg/kg. An RfD could be derived thusly:

$$\frac{14 \text{ mg/kg/day} \times 5 \times 1.0}{100 \times 10 \times 7} = 0.010 \text{ mg/kg/day}$$

where:

14 mg/kg/day* = NOAEL

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

1.0 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration)

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime.

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*Rounded from 14.4 mg/kg/day.

Varshavskaya (1968) studied the effects of CB in white rats. The animals were treated daily with solutions of CB in doses of 0.001, 0.01 or 0.1 mg/kg using a stomach tube. Controls received sunflower oil alone. The experiments lasted nine months. A variety of effects were seen at the two higher doses. None were seen at the lowest dose. While this study suggests that effects can be seen at very low doses when compared with the other studies, little in the way of experimental detail was presented in the published data. Therefore, it is difficult to assess fully the results presented, and one cannot conclude that this paper should be the basis for the development of an RfD for CB.

Knapp, et al. (1971) described the subchronic toxicity of CB in rats as reported in Hazelton (1967b). The animals were fed 12.5, 50, 100 or 250 mg/kg CB in their diets for 93-99 consecutive days. Male rats showed retarded growth at the highest dose level. At the two highest dose levels, a significant increase in liver and kidney weights was observed in females. However, no remarkable histological changes were noted. No effects were seen in the two lowest dose groups.

The results of this study suggest a NOAEL of 50 mg/kg/day. An RfD could be derived thusly:

$$\frac{50 \text{ mg/kg/day} \times 1.0}{100 \times 10} = 0.05 \text{ mg/kg/day}$$

where:

50 mg/kg/day = NOAEL

1.0 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration)

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime.

Knapp, et al. (1971) also described the subchronic toxicity of CB in dogs reported in Hazelton (1967a). The animals were given 27.25, 54.5, or 272.5 mg/kg CB orally by capsule 5 days per week for 93 days. Various toxic effects, including death, occurred with the high dose. Treatment-related histopathology in liver of mid-dose animals was evident. No observable effects were reported with the lowest dose.

The results of this study suggest a NOAEL of 27.25 mg/kg/day. An RfD could be derived thusly:

$$\frac{27 \text{ mg/kg/day} \times 1.0 \times 5}{100 \times 10 \times 7} = 0.02 \text{ mg/kg/day}$$

where:

27 mg/kg/day* = NOAEL

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

1.0 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for application to NOAEL in animals with no equivalent data in the human (longer-term exposure duration)

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime.

Subchronic studies were conducted in which both rats and mice were gavaged five times weekly with 0, 60, 125, 250, 500 or

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*Rounded from 27.25 mg/kg/day.

750 mg/kg CB in corn oil (10 animals/species/sex/dose level) (Battelle, 1978c,d). Food consumption did not vary among the groups in mice, but it was lower in the two highest dose rat groups. Body weight gain was affected in both species, with significant changes observed in male mice at all doses. In rats, significant changes occurred only at the two highest doses. No clinically significant CB-related changes were observed in any of the hematological parameters measured in either species. None of the clinical chemistry parameters measured in mice were changed. However, in the rats, SGPT and GGPT levels were elevated in two surviving females and one male at 750 mg/kg. Urinalyses of the controls and two highest dose groups revealed a dose-dependent polyuria with concomitant decreases in specific gravity and creatinine concentration. Also, at the two highest doses, urinary coproporphyrin excretion was increased in both sexes. In mice, this increase was observed only in females at 250 and 500 mg/kg. Liver:body weight ratios were increased significantly in mice of both sexes at 500 and 750 mg/kg. A slight increase was seen in male mice at 125 mg/kg. Both male and female rats at 250 and 500 mg/kg showed an increase. Mice and rats at the three highest doses (250, 500 and 750 mg/kg) all exhibited significant histopathological changes including hepatic necrosis, nephrosis, myeloid depletion, lymphoid depletion and lymphoid necrosis.

From these data, a NOAEL of 60 mg/kg/day can be identified. An RfD could be developed thusly:

$$\frac{60 \text{ mg/kg/day} \times 5 \times 1.0}{100 \times 10 \times 7} = 0.043 \text{ mg/kg/day}$$

where:

60 mg/kg/day = NOAEL, based upon increased liver:body weight ratios at higher doses

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

1.0 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration)

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime.

The only chronic exposure study of CB available is the NTP gavage bioassay in rats and mice (NTP, 1985). Both sexes of rats and female mice received 30 or 60 mg/kg/day. Doses were administered 5 days/week for up to 104 weeks.

Statistically significant changes in endpoints of toxicity included reduced survival in high-dose male rats and an increased incidence of reported neoplastic nodules in male rats. The results of this study indicate that a NOAEL for rats of 60 mg/kg/day can be identified.

If the rat data were used, an RfD could be derived thusly:

$$\frac{60 \text{ mg/kg/day} \times 5 \times 1.0}{100 \times 7} = 0.43 \text{ mg/kg/day}$$

where:

60 mg/kg/day = NOAEL from lifetime animal study

5/7 = conversion of 5 day/week exposure regimen to seven day/week continuous exposure

1.0 = ratio of administered dose absorbed

100 = uncertainty factor, appropriate for use with animal data and equivalent duration of exposure.

A two-generation reproduction study in rats exposed to chlorobenzene by inhalation has been reported by Nair et al., (1987). Parental generations were exposed for 10 or 11 weeks before mating and during mating, gestation and lactation until sacrifice soon after weaning of the last litter (females) or 3 to 4 weeks following mating (males). F_1 pups were selected for the F_1 parents on Day 21 after birth (weaning). Exposures were to 0 (control), 50, 150 or 450 ppm (0, 230, 690 or 2,070 mg/m^3) 6 hr/day, 7 days/week. Reproduction was not affected, but treatment-related pathology in mid- and high-dose animals was observed.

Using 50 ppm (230 mg/m^3) as a NOAEL, an RfD can be calculated as follows:

$$\frac{(230\ mg/m^3)(0.0093\ m^3/hr)(6\ hr/day)(0.5)}{0.35\ kg} = 18.3\ mg/kg/day$$

where:

230 mg/m^3 = NOAEL

0.0093 m^3/hr = assumed minute volume for a rat

6 hr/day = daily exposure period

0.5 = assumed absorption of agent

0.35 kg = assumed body weight for rat

$$RfD = \frac{(18.3\ mg/kg/day)}{100} = 0.2\ mg/kg/day$$

where:

18.3 mg/kg/day = NOAEL

100 = uncertainty factor, appropriate for application to NOAEL in animals with no equivalent data in the human (longer-term exposure duration).

The two-generation reproduction study in rats by Nair et al., (1987) is presented for comparison with the other studies considered for the Rfd calculation because it is the only available study of reproductive effects of CB. However, it is not considered for RfD development pertinent to drinking water since exposure was by inhalation, results were negative for reproduction and treatment-related pathology found with higher exposure levels was also looked for in other toxicity studies in rats treated orally with CB.

Existing Guidelines and Standards

EPA (1980) proposed ambient water quality criteria for chlorobenzene, one based upon available toxicity data (488 ug/L) and one based upon organoleptic effects (20 ug/L). These criteria were derived for the 70-kg adult, assumed to drink 2 liters of water per day and eat 6.5 g of contaminated fish and seafood per day. The toxicity-based criteria were calculated using the 14.4 mg/kg/day NOAEL from the study by Irish et al. (1963) and an uncertainty factor of 1,000.

ACGIH (1982) has adopted a TLV of 75 ppm (350 mg/m^3) for chlorobenzene in the workplace.

On the basis of a 1983 draft of the NTP report (NTP, 1985), the National Academy of Sciences performed a quantitative risk assessment to estimate excess lifetime cancer risk (NAS, 1983). The upper 95% confidence limit estimate of that risk was 2.13×10^{-7} per ug/L of drinking water. This corresponds to a drinking water concentration of 2.35 ug/L being equivalent to a 1 in a million excess risk.

Assumptions were defined for a 70-kg adult, drinking 2 liters of water per day.

WHO (1984) recommended a guideline for chlorobenzene of 3 ug/L based upon avoidance of taste and odor problems.

The current OSHA standard for monochlorobenzene is 75 ppm or 350 mg/m^3 (29 CFR 1910).

Quantification of Non-Carcinogenic Effects

The effects of CB in humans appear to be essentially the same as those observed in animal experiments. Several oral exposure animal studies are available in which acceptable dose-response data are described. The RfDs derived from these studies range from 0.01 mg/kg/day (Irish, 1963) to 0.43 mg/kg/day (NTP, 1985) (see Table VIII-1).

The fact that the RfD generated from the chronic studies is greater than those derived from the subchronic studies suggests that the 10-fold uncertainty factor used to estimate an RfD from subchronic data may be unnecessarily large. As can be seen when comparing the bioassay prechronic studies (Battelle, 1978c,d) with the chronic studies (NTP, 1985), the high doses used in the chronic studies differed from those required to produce frank tissue injury in the subchronic studies by factors of 2 to 4. Despite this relative closeness in doses, significant non-neoplastic lesions clearly attributable to chlorobenzene were not readily observed in the chronic study. On the other hand, one reason that the chronic study yielded a larger RfD than did the studies of lesser duration exposure may be that the "special" parameters

Table VIII-1. Possible RfDs for Chlorobenzene

Experiment	Reference	Possible RfD
subchronic gavage (rats)	Irish, 1963	0.010 mg/kg/day
subchronic dietary (rats)	Knapp, et al., 1971 (Hazelton, 1967b)	0.05 mg/kg/day
subchronic capsule (dogs)	Knapp, et al., 1971 (Hazelton, 1967a)	0.02 mg/kg/day
subchronic gavage (rats and mice)	Battelle-Columbus, 1978c,d	0.043 mg/kg/day
chronic gavage (rats and mice)	NTP, 1985	0.43 mg/kg/day

(i.e., urinalysis, clinical chemistry, hematology) were not included in the chronic study protocols. Had more sensitive indices of toxicity been evaluated in the chronic studies, effects may have been noted. At one or more of the doses administered. In light of this consideration, it may not be appropriate to suggest that the 10-fold uncertainty factor is too large.

Furthermore, the lowest RfD is considered to be that calculated using the dog study by Knapp et al. (1971) and Hazelton (1967a). The dog is considered to be the most sensitive species of those tested for chlorobenzene toxicity by the oral route because treatment-related effects were found at a lower dose in dogs. With respect to the Irish (1963) report, subsequent studies in rats along with the LOAEL in the Irish (1963) report being higher than that in the dog study support the RfD calculated using the dog study as actually the lowest of those calculated using oral exposure studies.

At this time, then, the subchronic study performed in dogs by Knapp et al. (1971) and Hazelton (1967a) provide appropriate data from which to derive the RfD for chlorobenzene. The RfD calculated with this study is close to those calculated with the subchronic studies in rats and mice. If these data were used to develop a DWEL, the DWEL would be derived as follows:

$$DWEL = \frac{70\ kg \times 0.02\ mg/kg/day}{2\ L} = 0.7\ mg/L$$

This DWEL assumes that the protected individual (the 70-kg adult) drinks 2 liters of water/day and that the sole source of exposure to chlorobenzene is via that drinking water.

The DWEL may be further reduced to take into account relative source contribution.

[It is important to note that the taste and odor threshold in water has been identified at levels ranging from 0.41 to 1.5 ug/l (Tarkhova, 1965) to 10 to 20 ug/l (Varshavskaya, 1967). Therefore, any Maximum Contaminant Level (MCL) for chlorobenzene may have to consider aesthetic, in addition to the toxic, consequences following exposure to CB in drinking water.]

Carcinogenic Effects

Chlorobenzene was tested for its carcinogenic potential in F344 rats and $B6C3F_1$ mice in the NTP Bioassay Program. A final report of the results of these studies is available (NTP, 1985). the experimental protocol is described on page VIII-17 of this document. With respect to carcinogenic responses, male rats dosed with CB exhibited a significant increase ($p < 0.05$) in the

incidence of animals with neoplastic nodules of the liver. There was no increase in incidence of hepatocellular carcinomas in male rats or of neoplastic nodules or hepatocellular carcinomas in female rats. However, there were hepatocellular carcinomas in two vehicle control male rats, and the combination of these data with those for neoplastic nodules results in the high dose males having a response of borderline significance ($p = 0.048$) by only one statistical test (life-table analysis). The report concludes that under the conditions of this bioassay, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in high-dose (120 mg/kg) male F344 rats, providing some but not clear evidence of carcinogenic activity of CB in male rats. Carcinogenic effects were not observed in female F344 rats or $B6C3F_1$ mice of either sex.

Quantification of Carcinogenic Effects

The National Academy of Sciences, in Volume 5 of Drinking Water and Health, using a linearized, non-threshold multistage model, estimated a risk and an upper 95% confidence estimate of lifetime risk in humans following exposure to chlorobenzene (NAS, 1983). The Academy derived an estimate of risk to exposure to a daily dose of 1 ug CB/liter of drinking water, based upon the limited evidence of carcinogenicity (liver neoplastic nodules in male rats). For a 70-kg adult drinking 2 liters of water per day, the estimated human lifetime risk at a daily dose of 1 ug/l = 3.71×10^{-8} and the upper 95% confidence estimate of lifetime cancer risk per ug/l = 2.13×10^{-7}.

EPA has classified chlorobenzene as to its carcinogenic potential, using the weight of evidence classification scheme in its risk assessment guidelines for carcinogens (U.S. EPA, 1986c). The Agency placed the chemical in Group D: Inadequate evidence. The Carcinogen Assessment Group in the EPA has derived no carcinogenic potency factor (q_1^*) or estimates of excess cancer risk on the basis of available data. The Science Advisory Board of the EPA has concluded that Group D (inadequate evidence in animals and humans) is an appropriate classification for chlorobenzene under the EPA guidelines.

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